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A STRUCTURAL INVESTIGATION OF
PORCINE GLYCERIDES

BY

CAROLINE GAIL STINSON

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "A Structural Investigation of Porcine Glycerides", submitted by Caroline Gail Stinson, B.H.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

"A Structural Investigation of Porcine Glycerides" was subdivided into three parts: (1) the fatty acid composition and glyceride structure of sow's colostrum, milk and body fat, (2) the fatty acid composition and glyceride structure of fat samples from piglets on a high fat diet; comparison of five sampling sites and (3) the identification of arachidic acid.

The glyceride structure of the sows' colostrum and milk fat followed the pattern of distribution established for porcine body fats and lard. There was a preferential positioning of palmitic acid at the 2-position in all the porcine glycerides, and oleic and linoleic acids were esterified primarily at the 1- and 3-positions. A comparison of the colostrum, milk and body fat revealed that colostrum resembled body fat in fatty acid composition and glyceride type distribution more than milk fat. Milk fat from the first week of lactation did not differ in fatty acid composition or glyceride type distribution from milk fat from the third week of lactation.

The fatty acid composition and glyceride type distribution of the piglet body fats varied with the site from which the sample was taken. The greatest difference was noted between kidney fat and the adipose tissue fats from the shoulder and loin. When comparing the outer and inner layers of the shoulder and loin, greater differences were found between samples taken from different layers at the same location, than were found between samples taken from the same layer but from different locations.

The presence of arachidic acid was identified in all of the porcine fat samples studied.

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INTRODUCTION

The elucidation of glyceride structure, which involves the position of component fatty acids on the glycerol molecule, is an active field of investigation in lipid research. The current theories on glyceride biosynthesis do not account for the specific distribution of fatty acids on the glycerol molecule, and only through extensive investigations of glyceride structure, and critical evaluation of the data obtained, can biosynthetic pathways which could account for this distribution be proposed and subsequently demonstrated. The purpose in carrying out the research contained in this thesis was to contribute to the information on the fatty acid composition and glyceride structure of porcine fats. It is hoped that this data will contribute to the vast amount of information necessary in the understanding of lipid metabolism.

REVIEW OF LITERATURE

Part I The Fatty Acid Composition and Glyceride Structure of Sow's Colostrum, Milk and Body Fat

Part II The Fatty Acid Composition and Glyceride Structure of Lipid Samples from Piglets on a High Fat Diet; Comparison of Five Sampling Sites

A. Methods of Determining Glyceride Structure

Natural fats are mixtures of mixed triglycerides having, in most cases, a wide variety of structures. If a fat contains n fatty acids, the number of possible glycerides, not including isomers, is $(n^3 + 3n^2 + 2n) \div 6$ (Lovern, 1955). If positional and stereo isomers are considered, the number of possible glycerides increases to n^3 . Since most natural fats contain at least five fatty acids, the determination of glyceride structure becomes a difficult task considering the number of theoretical possibilities.

Several methods have been developed for the determination of glyceride structure. These procedures break down the complex mixtures of glycerides into several simpler fractions, which can be further analyzed by gas-liquid chromatography

to determine their fatty acid composition. Often the methods of analysis are combined, to further decrease the complexity of the fat and provide practical information on its structure. The methods used in the analysis of glyceride structure will be outlined briefly.

1. Fractional Crystallization

Hilditch and Williams (1964), described this method thoroughly; using fractional crystallization in a solvent such as acetone, it is theoretically possible to divide a glyceride mixture into four types, S_3 , S_2U , SU_2 and U_3 , representing three, two, one and no saturated acyl groups, respectively, on the glycerol molecule. However, complex fats containing a wide range of fatty acids cannot be fractionated into the four glyceride types by this method.

2. Oxidation Procedures

Both Kartha (1953a) and Youngs (1961) have modified the original oxidation method of Hilditch and Lea (1927) for the determination of glyceride structure; this involves oxidation of all of the double bonds present in the triglycerides to yield saturated dicarboxylic half esters. With Kartha's method the unsaturated components in the fat are oxidized

by means of potassium permanganate in acetone and acetic acid, but the analysis is difficult and tedious, and the identities of the components are, in part, destroyed.

Youngs used a catalytic amount of KMnO_4 and an excess of periodate to carry out the oxidation, and because oxidation is more controlled than in the method of Kartha, the configuration of the molecules is not disturbed and good results are obtained. By combining this technique with that of pancreatic lipase hydrolysis, positional isomers of S_2U and SU_2 can be determined.¹

3. Countercurrent Distribution

In this method, (Hirsch, 1961; Scholfield, 1961), the substance to be fractionated is subjected to repeated partition between two immiscible liquid phases, thereby concentrating various fractions into one phase. The cost of the apparatus and the amount of work involved in the method make it unpopular.

1. Positional isomers of S_2U are $\text{E}_{\text{U}}^{\text{S}}$ and $\text{E}_{\text{S}}^{\text{S}}$; similarly the positional isomers of SU_2 are $\text{E}_{\text{U}}^{\text{S}}$ and $\text{E}_{\text{U}}^{\text{U}}$

4. Thermal Gradient Fractionation

This method (Magnusson and Hammond, 1959) involves the fractionation of a sample in a solvent on a column which has a linear thermal gradient between bottom and top. The most soluble fractions are eluted first, as the others crystallize out on their way down the column.

5. Thin-Layer Chromatography

Privett and Blank (1961) have developed a method involving ozonization of the double bonds present in the glycerides. The ozonides are reduced, and the "aldehyde cores" are separated by thin-layer chromatography and analyzed quantitatively by densitometry. A component analysis of the spots is accomplished by gas-liquid chromatography.

Kaufmann and Wessels (1964) have employed reversed phase chromatography for the qualitative separation of triglycerides in some natural fats.

Triglyceride separations can also be obtained by adsorption chromatography on silver-nitrate impregnated silica plates (Barrett et al., 1963). These separations are based on the degree of unsaturation, and not on the molecular weight of the fractions.

6. Gas-Liquid Chromatography

McCarthy and Kuksis (1964) have combined adsorption, partition and gas-liquid chromatography to elucidate the glyceride structure of several natural fats. Gas-liquid chromatography of methyl esters of the component fatty acids reveals the fatty acid composition of the sample, and gas-liquid chromatography of the triglycerides results in a quantitative analysis of each glyceride type present in the sample, on the basis of carbon number or molecular weight.

7. Hydrolysis with Pancreatic Lipase

The pancreatic lipolysis technique, employed in the study of glyceride structure, is based on the demonstration that pancreatic lipase (E.C. 3.1.1.3) specifically hydrolyzes the fatty acids esterified with the external hydroxyl groups of glycerol (Mattson and Beck, 1956; Savary and Desnuelle, 1956). Because this method was used by the author for studies on porcine glycerides, this technique will be reviewed in more detail.

(a) Characterization of Pancreatic Lipase

The Commission on Enzymes of the International Union of Biochemistry (1961), has systematically named pancreatic lipase, glycerol ester hydrolase, thus differentiating the action of this enzyme from the group of enzymes known as esterases. Pancreatic lipase attacks glycerol esters readily (Desnuelle, 1961; Dixon and Webb, 1964), and although this enzyme will hydrolyze methyl esters, the action is quite slow. The physical state of the substrate is considered as the differentiating feature between esterases and lipases (Desnuelle, 1961), as pancreatic lipase acts only on emulsions of insoluble esters, and esterases act only on molecules in solution. Since the biological substrates for lipases are highly insoluble in water, and lipases themselves are water soluble, the enzyme action takes place at the oil-water interface (Sarda and Desnuelle, 1958).

Porcine pancreatic lipase can be purified (Marchis-Mouren et al., 1960) from an aqueous extract of pancreatin by a series of fractionations with ammonium sulfate and acetone in a cold atmosphere, selective absorptions on tricalcium phosphate and aluminum hydroxide and finally zone electrophoresis of long duration at pH 5.25 in starch columns. The enzyme obtained is purified 135 fold, is electrophoretically and chromatographically homogeneous, and could therefore be

considered pure. Most of the glyceride analyses carried out, however, use a crude preparation of the enzyme without further purification.

(b) Optimal Conditions for Hydrolysis

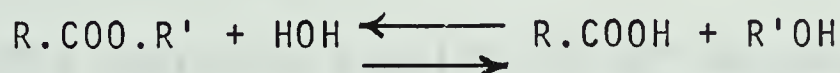
Since pancreatic lipase action occurs at the oil-water interface, it is desirable to work with a fine emulsion exposing as much surface area as possible. A fine emulsion is produced in most cases, by the addition of bile salts to the hydrolysis mixture and rapid agitation (Mattson and Volpenhein, 1961); the presence of bile salts is also necessary for the activation of the enzyme (Fritz and Melius, 1963).

An optimum temperature of 37 C and an optimum pH of 8 should be maintained throughout the hydrolysis (Desnuelle et al., 1948). Other conditions for the best activity of the enzyme include high ionic strength (Mattson and Beck, 1955), and the presence of calcium ions (Constantin et al., 1960). Calcium ions are believed to remove inhibiting free fatty acids; if calcium ions are omitted, diglycerides accumulate, and the reaction does not proceed to the formation of significant amounts of monoglyceride. The importance of the formation of monoglycerides will be discussed later.

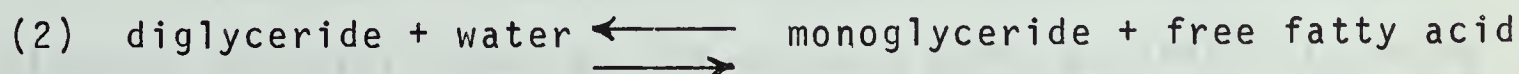
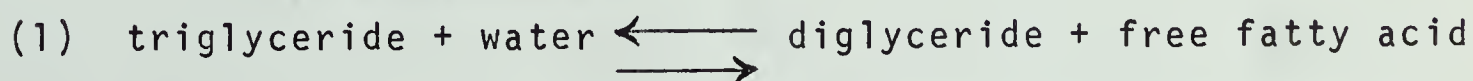
As lipolysis proceeds, the liberation of free fatty acids lowers the pH of the medium considerably, and pancreatic lipase activity falls sharply under acid conditions (Schonheyder and Volqvartz, 1945). The maintenance of pH 8 can be accomplished by one of two methods: (1) if the amount of triglyceride used is small, a relatively large amount of buffer in the medium will stabilize the pH, (2) the continuous addition of alkali, not only maintains the desired pH, but also serves as a check on the extent of hydrolysis (Coleman, 1965).

(c) Mode of Action

Pancreatic lipase catalyzes the breakdown of an ester into its acidic and alcoholic components as shown in the following equation (Hanahan, 1960):



When glycerides are the substrates, the fatty acids are removed one at a time as follows:





This is a stepwise reaction, and if completed, the products will be glycerol and three free fatty acids. The hydrolysis rate decreases in the order: triglycerides > diglycerides > monoglycerides, and the rate of lipolysis of monoglycerides to glycerol is so slow that unless the amount of enzyme and emulsifier is present in a large excess, and the time is long enough, this step will not take place (Desnuelle and Savary, 1963). It should be noted that the hydrolysis of monoglycerides to glycerol and free fatty acids is irreversible (Borgström, 1964).

(d) Specificity

(i) Substrate

The substrate specificity of pancreatic lipase has been discussed previously under (a). True lipases act only in heterogeneous systems.

(ii) Positional

Pancreatic lipase has a definite specificity for the cleavage of ester bonds at the one and three positions (primary

positions) of the glyceride molecule (Borgström, 1953; Mattson and Beck, 1956; Savary and Desnuelle, 1956). By working with synthetic triglycerides, these authors have confirmed that the breakdown of triglycerides occurs as follows:



Experiments have shown that some 1-monoglycerides are also formed during hydrolysis, probably because of acyl migration of the fatty acid from the internal position on the glycerol molecule to an external position. If hydrolysis is carried out in as short a time as possible under the optimal conditions described earlier, acyl migration should not be a problem with most fats studied. Acyl migration can also occur, however, in subsequent treatments, such as purification and separation by chromatography.

(iii) Fatty Acid

The pancreatic lipolysis technique has been found to be unsuitable when determining the structure of glycerides containing either short chain fatty acids (C_4-C_{10}), (Boudreau and de Man, 1965), or fatty acids with more than three double bonds (Brockeroff et al., 1966).

When short chain fatty acids are on the external positions of the glycerol molecule, the positional specificity of the enzyme is enhanced. When the triglyceride substrate contains short chains bound to the inner position, high proportions of originally inner chains appear in the free fatty acid fraction following hydrolysis (Entressangles et al., 1961; Clément et al, 1962; Boudreau and de Man, 1965). A possible explanation for these results is that short chain fatty acids isomerize at an exceptionally high rate (Entressangles et al., 1964). It can be seen that if one of the outer chains is removed during lipolysis, the inner short chain fatty acid could migrate to an outer position, and once there, would be readily hydrolyzed.

(iv) Stereochemical

Tattrie et al., (1958) found the enzymatic hydrolysis of the primary ester groups of glycerides to be non-stereospecific.

(v) Specificity for cis or trans Isomers

The possible influence of trans fatty acids on either the fatty acid specificity or positional specificity of pancreatic lipase was studied by Jensen et al. (1964).

Glycerides containing elaidic acid (trans-9-octadecenoic acid) were hydrolyzed, and the enzyme did not appear to differentiate between elaidic acid and its cis-isomer, oleic acid.

(e) Use as a Method for Determining Glyceride Structure

Since pancreatic lipase specifically hydrolyzes ester bonds at the external positions of the glycerol molecule, fatty acid analysis of the monoglycerides formed during hydrolysis will reveal the fatty acids esterified at the internal position of the glyceride molecule. The other acids, present in the original fat, are assumed to be preferentially esterified to the external positions. No differentiation is made between the two external positions (#1 and #3) in the analysis. If one is allowed to assume that acids in the 1-and 3-positions are randomly distributed, then pancreatic hydrolysis permits the most detailed analysis of natural fats so far reported, an important point in this connection being the fact that isomeric triglycerides are distinguished by this method (Coleman, 1963).

8. Stereospecific Analysis

Brockerhoff (1965) developed a method to differentiate between the fatty acid composition at the 1- and at the 3-positions of glycerides. This method has been based on the use of pancreatic lipase to determine the fatty acids at the internal and external positions, but recently methyl magnesium bromide has been used (Yurkowski and Brockerhoff, 1966). Hydrolysis with methyl magnesium bromide has been reported by these authors to be random, and the partial glycerides necessary for further analysis are isolated by thin-layer chromatography.

B. Theories on Glyceride Distribution

The theories on glyceride distribution are described to provide a general description of the structures which have been found experimentally, and to form a basis for interpretation of experimental results (Coleman, 1963).

1. Even Distribution

One of the earliest theories was that of Collin and Hilditch, (1929), in which they proposed that fatty acids were evenly distributed on the glycerol molecules. Although the

theory gave a fair approximation to experimental results for a large number of vegetable fats, there were marked departures from it when saturated animal fats were examined.

2. Random Distribution

This theory assumes that fatty acids are distributed both between the triglycerides and within each triglyceride molecule randomly. Most pancreatic lipase data have revealed that fats are not randomly distributed.

3. Restricted Random Distribution

This theory was proposed by Kartha (1953b) to account for the discrepancy noted between the proportion of trisaturated glycerides expected on the basis of a purely random distribution of fatty acids, and the proportion of trisaturated glycerides found experimentally.

4. 1,3 Random, 2 Random Theory

This theory (Vander Wal, 1960; Coleman and Fulton, 1961) proposed after the technique of pancreatic lipase analysis had been developed, assumes that the fatty acids found in the monoglycerides after pancreatic lipolysis, represent the fatty acids occupying the 2-position of the original

triglyceride. The remaining acids are distributed randomly amongst the 1- and 3-positions. Calculations based on the proportions of unsaturated and saturated fatty acids, and the mole percent fatty acid composition of the sample, result in a structural analysis which gives the amounts of isomeric glycerides as well. Previous theories were based completely on mathematical principles, but this theory is the first to give any consideration to the biosynthetic mechanisms involved in glyceride synthesis. It represents the most satisfactory interpretation of pancreatic hydrolysis data available, and a great deal of experimental evidence has been reported in its favour (Youngs, 1961; Subbaram and Youngs, 1964; Privett et al., 1965).

C. Fatty Acid Composition and Structure of Porcine Glycerides

1. Fatty Acid Composition

The major fatty acids present in porcine glycerides are: oleic (octadecenoic acid), palmitic (hexadecanoic acid), stearic (octadecanoic acid) and linoleic acid (octadecadienoic acid); minor fatty acids are myristic (tetradecanoic acid) and palmitoleic acid (hexadecenoic acid).

2. Glyceride Structure

When pancreatic lipase was first used to determine the general distribution patterns of fatty acids in triglycerides of natural fats, it was found that the patterns were not only non-random, but also that stearic acid and to a lesser degree, palmitic acid, tended to occupy the external positions of the glycerol molecule, while linoleic acid and often oleic acid accumulate in the internal position of most natural fats. The fats of pigs and marine mammals are two striking exceptions to the general pattern. Early investigations (Hilditch and Stainsby, 1935; Quimby et al., 1953), verified by later work (Mattson and Lutton, 1958; Savary and Desnuelle, 1959; Coleman, 1961), report the predominance of palmitic acid in the 2-position of lard. Mattson et al. (1964) investigated the distribution of fatty acids in the triglycerides of the Artiodactyla (even-toed animals) of which the pig is a member. It was found that the unusual distribution pattern of the pig is also found in the wild boar and peccary but not the hippopotamus; these authors discussed the evolutionary aspects of this distribution.

D. Effect of Sampling Site on Fatty Acid Composition and Glyceride Structure

1. Body Tissue Lipids

Most of the work on porcine glycerides has been carried out on either the depot fat of the pig or commercial lard. Lard could include both body fat and fat rendered from organ tissues, and for this reason, Privett et al. (1965) suggested that analyses carried out on such processed fats will not provide the extensive information required for the study of triglyceride metabolism.

Anatomical variation in fatty acid composition and glyceride structure has not been studied extensively, probably because up until a few years ago, analytical methods were not available for the investigation of small samples.

(a) Variation in Fatty Acid Composition

Hilditch and Williams (1964) reported variation in the fatty acid composition of the trisaturated glycerides of pork fat obtained from different sites within the animal. These findings have been supported by both Leat et al. (1962) and Sink et al. (1964) in studies on swine. Leat et al. found

that inner back fat contained more stearic and palmitic acids and less oleic and linoleic acids than outer back fat; inter-muscular fat was similar to inner back fat, while perinephric fat was more saturated. Sink et al. concluded from their studies that saturated fatty acids are preferentially deposited in perirenal tissues as compared to the subcutaneous area, and in this latter area, saturated fatty acids are deposited in the inside layer rather than the outside layer.

Reports on human depot fat (Hegsted et al., 1962; Imaichi et al., 1965) show that there are significant differences in fatty acid composition of adipose tissue from several sites. Most investigators speculated that these differences were created to adjust the physical property of adipose tissue to the environmental temperature; tissue temperatures of thinly insulated extremities are lower than at body center. Fatty acids which were significantly different from site to site, were those which could be synthesized in the human body. Linoleic acid, which is not likely to have been synthesized in the body, did not show any meaningful difference.

(b) Variation in Glyceride Structure

Savary et al. (1957) found that although fatty acid

composition changes with the anatomical location of the pork sample, the preferential positioning of palmitic acid at the 2-position was unaffected. Mattson et al. (1964) reported that both subcutaneous and peritoneal fat from the Panamanian peccary had essentially the same fatty acid composition and distribution. Privett et al. (1965), when studying the glyceride structure of various rat tissues, however, noted a preferential incorporation of certain fatty acids into specific positions, resulting in different structures of the triglycerides in various tissues. Barford et al. (1965) found that the distribution of the principle glyceride types (S_3 , S_2U , SU_2 and U_3) in the internal and external adipose tissue fats from the same pig was non-random and the percentages of palmitic acid at the 2-position in these adipose fats were comparable. However, liver glycerides from this same animal differed strikingly from adipose glycerides; the liver glycerides of lamb, rabbit and dog also differed considerably from adipose glycerides in distribution and in percentages of individual fatty acids in the 2-position. A recent study by Chacko and Perkins (1965) on the glyceride type distribution of pork fat samples showed definite variations according to the area from which the fat was obtained. Back fat contained a higher proportion of SSU than fats taken from other positions; perinephric fat contained considerably less U_3 than did fat from other sample sites.

2. Milk Lipids

Although a great deal of information is available on bovine milk fat, and to a lesser extent, human milk fat, very little has been published on sow's milk fat. Among the more extensive investigations which have included fat analyses of sow's milk and colostrum are those of Braude et al. (1947), Bowland et al. (1949a,b) and Perrin (1954). Sheffy et al. (1952), after studying the fat constants and phospholipid content of sow's milk, concluded that the fatty acid composition of sow's milk fat and lard are similar. The component fatty acids of sow's milk fat were investigated in 1944 by de la Mare and Shorland, and in 1963 de Man and Bowland determined the fatty acid composition of sow's milk fat by gas-liquid chromatography. The fatty acid composition of sow's colostrum differed to a lesser extent from the sow's backfat than did the milk fat. As with ruminants, the composition of the milk fat of the sow was found to be more complex than the depot fat, in that the milk fat contained small amounts of capric (decanoic acid) and lauric acids (dodecanoic acid) and greater amounts of unidentified fatty acids. Ramaiah (1965) compared the milk fats of ruminants and nonruminants using thin-layer chromatography and gas-liquid chromatography. He found that short chain fatty acids were absent from triglycerides and phospholipids of the

nonruminants' milk fat, in contrast to the milk fat of ruminants.

The presence of appreciable amounts of short chain acids in most milk fats makes it difficult to analyze their glyceride structure by the pancreatic lipase method. Sow's milk fat, however, which contains no short chain fatty acids, is suitable for examination by pancreatic hydrolysis. Coleman (1965) has presented data on the glyceride composition of sow's milk fat, which indicate that porcine milk fat has a glyceride structure similar to that of the body fat in that there is a predominance of palmitic acid in the 2-position.

When comparing the fatty acid composition and glyceride structure of depot fat and milk fat of various animals, Freeman et al. (1965) noted that both the amount of unsaturated fatty acids in the triglycerides and the proportion of these acids esterified at the 2-position are higher in the depot fat than in the milk fat of the same species. The data for pig depot fat corresponded closely to the results obtained for human milk fat; data on the glyceride structure of sow's milk fat were not given in the paper.

E. Influence of Dietary Fat on Fatty Acid Composition and Glyceride Structure

1. Metabolism of Lipids

Lipids are digested in the intestine, and absorption takes place mainly in the jejunum (Dawson and Isselbacher, 1960; Wilson, 1962; Wiseman, 1964). During digestion, the hydrolytic action of pancreatic lipase breaks down fats into 2-monoglycerides, free fatty acids and glycerol; this precedes absorption into the intestinal mucosa which is accomplished by the formation of micelles made up of monoglycerides, free fatty acids and bile salts (Hofmann and Borgström, 1962; Johnston and Borgström, 1964). It is within the intestinal mucosa that differentiation occurs between long and short chain fatty acids. The esters of the shorter chain fatty acids are rapidly hydrolyzed by the intermucosal monoglyceride lipase; the resulting free fatty acids are not readily reesterified and are removed by the portal circulatory system to the liver where they predominantly are oxidized to 2-carbon fragments (Senior, 1964; Lands, 1965). Long-chain fatty acids, and monoglycerides containing these acids, are resynthesized in the intestinal mucosa to triglycerides by two major metabolic routes. One pathway involves the acylation of fatty acids to acyl-CoA thioesters which then react with L- α -glycerophosphate

to yield phosphatidic acid derivatives. The other route involves the direct interaction of monoglycerides with fatty acyl-CoA molecules to yield diglycerides and triglycerides (Isselbacher, 1965). The latter route appears to be the major pathway of di- and triglyceride synthesis during fat absorption (Senior, 1964). The triglycerides are then incorporated into discrete particles called chylomicrons, which enter the general circulatory system via the thoracic duct (Borgström, 1960). Dietary fat that enters the blood as chylomicron triglyceride, goes in part to the fat depots and utilizing tissues, and in part to the liver, where the chylomicron components are removed as intact molecules (Belfrage et al., 1965). In the liver, the triglyceride ester bonds are disrupted, and the liberated fatty acids are utilized for oxidation or synthesis of fatty acid esters, which can be recirculated to extrahepatic tissues (Robinson, 1963). Benson and Rampone (1966) have recently reviewed the gastrointestinal absorption of lipids.

2. Modification of Dietary Lipids

Throughout the course of fat metabolism, especially during absorption and deposition, many opportunities exist for the alteration of dietary lipids to a composition and structure specific for the particular animal involved. These

aspects will be discussed individually.

(a) Alterations in Fatty Acid Composition

(i) During Absorption

Bayley and Lewis (1965) studied the digestibility of various fats and fatty acids in pig feeding and concluded that the absorbability of a particular acid is to a large extent influenced by the other acids in the fat. On the other hand, Cook and Thomson (1951) found a species variation in the absorbability of fats by demonstrating that olive oil was not absorbed to the same extent in the various species of animals studied. Digestion trials of Sewell and Miller (1965) revealed a highly significant increase in total lipid digestibility of a corn oil diet in comparison to diets containing lard or tallow. Stearic acid had a lower absorbability than other fatty acids present, and palmitic acid was more poorly absorbed than oleic and linoleic acid. Renner and Hill (1961) found that in the chick, fatty acids were not absorbed to nearly the extent when fed as free fatty acids as when fed in the form of glycerides.

Considerable dilution of dietary fat takes place in the lumen by endogenous lipids (supplied from within the animal). Investigations by Bragdon and Karmen (1960), revealed

that during the period following a meal of either corn or coconut oil, the fatty acid composition of the lymph chylomicrons rapidly changed toward the composition of the diet and eventually became almost identical with it. It was found, however, when lymph samples were collected at later stages that the fatty acid composition of the chylomicrons did not closely resemble that of the diet fed, and substantial amounts of non-dietary fatty acids were present (Karman et al., 1963); 43% of the chylomicron triglyceride in the lymph samples collected at later stages came from a source within the animal. It appeared that the greater the amount of exogenous lipid in the lymph, the greater also was the amount of endogenously supplied lipids.

(ii) During Deposition

Alterations in dietary fatty acid composition occur when fatty acids are taken up and released from animal cells at different rates, oxidized at different rates, and incorporated into various tissues and lipid fractions in different proportions. Göransson (1965a-g), and Göransson and Olivecrona (1964, 1965) have conducted a series of experiments on the metabolism of several fatty acids in the rat, and obtained results which indicate that in general, fatty acids disappear more rapidly into the cells with increasing unsaturation and

decreasing chain length. Knittle and Hirsch (1965), however, found that adipose tissue was able to esterify long chain free fatty acids more readily than acids with less carbon atoms. Most of the free fatty acids containing less than eight carbon atoms were elongated to acids with sixteen to eighteen carbons which were esterified more readily. It has been found (Möhrhauer and Holman, 1963) that dietary linolenate inhibits the conversion of linoleate to arachidonate, and that increasing amounts of dietary linoleate suppresses the levels of linolenic acid metabolites (Rahm and Holman, 1964)

Dietary fatty acids can be altered further by tissues synthesizing acids de novo, or carrying out inter-conversions to other fatty acids, or both. Lipogenesis from dietary carbohydrate which is in excess of metabolic needs occurs mainly in adipose tissue and the liver (Robinson, 1963). Palmitic acid is the main fatty acid formed from small molecule precursors by animal tissue preparations and is accompanied by smaller amounts of myristic and stearic acids. In mammary gland preparations, intermediate fatty acids ranging from C_8 - C_{12} are also formed to an appreciable extent (Carroll, 1965). Odd numbered fatty acids and branched-chain fatty acids are found in small amounts in many animal tissue lipids and these can be synthesized completely from small

molecule precursors. Elovson (1965) has shown that in the rat, chain shortening of stearic acid occurs by eliminating one acetate unit from the molecule, which results in the formation of palmitic acid. Craig and Rogers (1965) have demonstrated degradation of erucic acid to arachidic and oleic acids in the same animal. The major monosaturated fatty acids in animal tissue, palmitoleic and oleic acids, appear to be formed largely from palmitic and stearic acid respectively (Carroll, 1965; Elovson et al., 1965). Two common transformations which occur in animal tissue are: (1) elongation by addition of 2-carbon units to existing fatty acids and (2) chain elongation of unsaturated fatty acids with insertion of additional double bonds between the carboxyl group and the existing double bonds. The latter pathway gives rise to families of polyunsaturated fatty acids in which members of each family have the same configuration at the methyl end of the chain but differ in chain length, or total number of double bonds, or both (Long, 1961; Klenk, 1965).

In spite of the number of possible alterations, it has been found that animals fed on rations containing relatively large proportions of fat, will more or less directly assimilate much of the dietary fat, and the composition of the resulting depot fats is affected to a considerable extent according to the nature and amount of fat ingested by the

animal (Hilditch and Williams, 1964). It is generally accepted that the inclusion of a greater number of unsaturated fatty acids in the dietary fat causes an increase in the iodine number of the depot fat of the animal (Greer et al., 1965; Howard et al., 1965). Several workers (Widmer and Holman, 1950; Hill et al., 1961; Leat et al., 1962; Borgman, 1964; Chung et al., 1964; Mattson et al., 1964) have shown that the linoleic acid content of the pig and other animal tissues was greater with a higher linoleic acid intake. After feeding pigs a diet rich in cod-liver oil and lard, Garton and Duncan (1954) concluded that these fats were absorbed essentially unchanged and were deposited additively along with typical "synthesized" pig fat in the depots. Blummer et al. (1957) noted that the fat of hogs fed a diet containing soybean oil was soft, but when coconut oil replaced the soybean oil in the last stages of fattening, the iodine number of the depot fat decreased, with an increase in hardness of the fat. Christensen (1963) observed that most of the dietary coconut oil was modified before deposition in the back fat of bacon pigs, whereas soybean oil was deposited without alteration.

Marion and Woodroof (1963) analyzed the fatty acid composition of various tissues of chicken broilers being fed different dietary fats. Each tissue analyzed responded to

the dietary fats by tending to assume the fatty acid composition of the fat in the diet. Chung et al. (1965) examined the tissues of pigs fed whole peanuts, and found that significant changes in fatty acid composition occurred primarily in the organ tissues as compared to body tissues. Of the three organs studied, heart, liver, and kidney, the heart was the least prone to fatty acid changes. In a study by Hill et al. (1961), heart and liver tissues increased in polyunsaturated fatty acid concentrations when pigs were fed linoleate-supplemented diets. Chung and Lin (1965) observed that when a diet high in linoleic acid was given, greater proportions of this acid were deposited in all of the tissues studied. With a higher oleate diet, greater proportions of oleic acid were deposited in the tissues studied with the exception of the heart and chop. Only small proportions of lauric and myristic acids were present in all the tissues studied although large proportions were consumed.

Dahl and Persson (1965) have suggested that in rapidly formed fat, such as milk fat, more of the character of the dietary fat is reflected. de Man and Bowland (1963) studied the milk fat of sows receiving a high fat diet of stabilized tallow and found that the milk contained on the average more oleic acid and less palmitic, palmitoleic, and linoleic acids than the milk fat of sows on a standard ration.

There was also less lauric acid and myristic acid, but these two acids were not of quantitative significance in the milk fat. When high fat diet was given, the same differences were found in the back fat as were reported for the milk fat.

(b) Alterations in Glyceride Structure

(i) During Absorption

Very little is known about the manner in which the positions of the fatty acids of the glycerol molecules of dietary fat affect the structure of the final glycerolipid. As mentioned previously, fats are absorbed as free fatty acids and monoglycerides, most of the monoglycerides as 2-isomers. Clark and Hübscher (1960) proposed that the monoglycerides could serve as the backbone for the resynthesis of triglycerides by the intestinal mucosa. Savary et al. (1961) and Mattson and Volpenhein (1962, 1964) found, upon examination of chylomicron triglycerides in the lymph, that the absorbed 2-monoglyceride structure was retained in the newly-formed triglycerides. From this observation Savary et al. concluded that digestion and absorption are not able to entirely abolish the structure of the ingested triglycerides. Brockerhoff et al. (1964) fed a 2-labelled

triglyceride, glyceryl-1,3-dioleate-2-palmitate-1-C¹⁴ to lobster, cod, trout and rat and observed that the invertebrate and fish retained 50-80% of the original structure after absorption and deposition of the fat. No retention was noted in the triglyceride of the liver and carcass of the rat. Clément et al. (1965a) investigated the fatty acid composition and glyceride structure in the intestinal lumen, mucosa, lymphatic chylomicrons and perirenal adipose tissue of rats after administration of a diet containing 10-20% coconut oil. In coconut oil, most of the lauric acid is located in the 2-position of the triglyceride. These authors found that the proportion of lauric acid in the 2-position in the triglyceride decreased progressively from the intestinal lumen towards the adipose tissue where it predominated in the external position.

(ii) During Deposition

Loriette et al. (1963) demonstrated that when rats and pigs were fed rations containing elaidinized peanut oil, the trans fatty acids were found predominantly in the external positions of the depot triglycerides. This positional specificity occurred in spite of the fact that the distribution of saturated and unsaturated acids between the internal and external positions is different in these two species.

Clément et al., (1965b) found that endogenous oleic acid was placed in the 2-position after absorption, while exogenous oleic acid was found in the external positions as in the case of elaidic acid. They concluded that a fatty acid is placed on an external position, not because of preference for this position, but because it has less affinity for the 2-position than do other acids.

Privett et al. (1965) in carrying out an extensive study of the influence of dietary fat on the triglyceride structure in the rat, found little resemblance between the structures of the triglycerides of the diet and the structures of the triglycerides from the tissues. These results were supported by Flanzy et al. (1965) in a glyceride structure study involving swine. The fatty acid composition and glyceride structure of the porcine depot fat was affected only by the fatty acid composition of the dietary fat and not by the structure of the corresponding glycerides. Although retention of dietary glyceride structure has not been demonstrated in mammals, partial retention of the fatty acids in the 2-position as mentioned earlier, has been demonstrated in the lobster, cod and trout (Brockerhoff et al., 1964). It has been hoped (Brockerhoff et al., 1966) that the pattern of distribution in relation to the pattern of dietary glycerides would give some indication of how the fat was synthesized.

Part III Identification of Arachidic Acid

Several authors (Flanzy et al., 1965; Sewell and Miller, 1965) have been concerned only with the major fatty acids present in lard, and others (Youngs, 1959; Coleman, 1961; Privett and Blank, 1961; Youngs, 1961; Subbaram and Youngs, 1964) have analyzed lard on the basis of glyceride types present. Studies which have included minor fatty acids have left some confusion as to the positive identification of these acids. This author concerned herself particularly with the separation and identification of linolenic (octadecatrienoic acid) and arachidic acid (eicosanoic acid).

Subbaram and Youngs (1964), Mattson et al. (1964), Chacko and Perkins (1965) and Brockerhoff et al. (1966), all reported small amounts of linolenic acid in lard triglycerides. Sink et al. (1964) observed a variation in linolenic acid content from 0.69% to 1.35%, depending upon the site from which the fat sample was taken. Chung and Lin (1965) reported the presence of small amounts of both linolenic acid and gadoleic acid (eicosenoic) in pork samples. In a study of the component fatty acids in lard, Herb et al. (1963) and Magidman et al. (1963) have presented information on the identification of the minor and trace fatty acids.

These workers used a polar and nonpolar gas-liquid chromatographic column for the identification. Fractions were collected from the nonpolar silicone column and rechromatographed on a polar ethylene glycol succinate polyester column. These authors reported the presence of 0.5% linolenic acid, 0.4% arachidic acid and 0.9% gadoleic acid.

Iverson et al. (1963) have reported that the methyl esters of linolenic acid and arachidic acid are difficult to separate on a diethylene glycol succinate column at 200 C, but if the temperature is lowered, partial resolution is accomplished. Supina (1963) stated that in the analysis of fatty acid methyl esters with ethylene glycol succinate polyester, overlapping of linolenate and arachidate peaks occurs. Complete separation of these can be obtained with ethylene glycol adipate polyester, but at a sacrifice of the separation between methyl stearate and methyl oleate.

EXPERIMENTAL PROCEDURE

PART I The Fatty Acid Composition and Glyceride Structure of Sow's Colostrum, Milk and Body Fat

A. Source of Experimental Material

Colostrum, milk and body fat samples were taken from each of four Yorkshire sows raised at the University of Alberta farm on standard diets. The four sows, designated as below, farrowed on the following dates:

230X - November 3, 1965

50 - November 8, 1965

230XX - November 11, 1965

20 - November 18, 1965

Colostrum samples were taken one to two hours after farrowing, and milk samples were taken after one and three weeks of lactation; milk let-down was induced by the injection of ten international units of oxytocin (Pitocin, Parke-Davis and Co.) (Bowland et al., (1949c). Body fat samples were obtained by biopsy on the following dates:

230X - November 17, 1965

50 - November 17, 1965

230XX - November 24, 1965

20 - November 24, 1965

Both general and local anesthetics were administered prior to the removal of representative samples from the right shoulder of each sow.

The lipids from the sows' diet were also analyzed. Before farrowing, the sows were on a standard gestation ration, and after farrowing, they were fed a lactation ration. The composition of these basal swine rations is given in Table 1.

B. Extraction and Preparation of Lipids from Colostrum, Milk, Body Tissue and Feed Samples

1. Colostrum and Milk Samples

The colostrum and milk samples were shell frozen in large freeze-dry flasks using dry ice-acetone (-70 C) and lyophilized on a Virtis freeze drying apparatus (Virtis Research Equipment, Gardiner, N.Y.).

The resulting powder was ground using a mortar and pestle, and then transferred into Soxhlet thimbles (43 mm x 123 mm). The lipids were extracted from the powder in a Soxhlet apparatus with light petroleum ether (bp 30-60 C) during a 24 hour period. The petroleum ether was evaporated on a steam bath and the samples were dried at 60 C under

Table 1. Compositions of basal swine rations percentage.

Ingredients	Gestation Ration	Lactation Ration	20% Stabilized Fat Diet
	100 lb.	100 lb.	
Wheat	10.00	10.00	31.75
Barley	9.95	23.10	
Oats	40.00	35.00	20.0
Stabilized fat			20.0
Soybean meal	5.00	5.00	22.0
Linseed meal	2.00	2.00	
Fishmeal		2.00	3.0
Meat meal	6.00	6.00	
Alfalfa meal	15.00 ^b	5.00	2.0
Wheat bran	10.00	10.00 ^c	
Iodized salt	0.50	0.50	
Ground limestone	0.80	0.80	0.5
Dicalcium phosphate or bonemeal	0.50	0.30	0.5
Trace mineral mixd			0.1
Vitamin mix (249C) ^d	0.05	0.10	0.05
Vitamin B12 (9 mg./lb.)	0.05	0.05	0.05
Vitamins A and Da	+	+	+
Feeding oil (1000 A, 150 D)	0.15	0.15	

- a. 2,000,000 I.U. of A and 400,000 I.U. of D per ton for grower
 - b. 4,000,000 I.U. of A and 800,000 I.U. of D per ton for sow rations
 - c. 10% of the alfalfa meal was removed and replaced with oats May 15- September 15 when sows were on pasture.
 - d. 1 lb. of bran per day extra was used from the time the sows were placed in farrowing stalls until several days after farrowing.
- Composition may be obtained from the Department of Animal Science, University of Alberta.

vacuum. The percentage of lipid was expressed on a dry weight basis. Samples were stored at -20 C until used for analysis.

2. Tissue Samples

These samples were frozen and lyophilized as described under 1. The sample was cut into small pieces, placed in Soxhlet thimbles (33 mm x 80 mm), and extracted, treated and stored as described under 1.

3. Feed Samples

The extraction method used was similar to that of Drury and Treadwell (1953). The dried, ground samples were refluxed three times with an appropriate volume of 95% ethyl alcohol and then refluxed three times using anhydrous diethyl ether. Each refluxing was carried out for one hour, at the end of which, most of the liquid was decanted off and filtered using a Büchner funnel. The ethanol extracts were combined and evaporated under vacuum at 50 C, and the ether extracts were evaporated on a steam bath. The residues from both solvent extractions were combined and the lipids extracted with light petroleum ether. The extract was washed with water, dried over sodium sulfate, and the petroleum ether evaporated on a steam bath. The samples were dried under vacuum at 60 C and stored at -20 C.

C. Pancreatic Lipase Hydrolysis

Pancreatic lipase, (E.C. 3.1.1.3) (Steapsin, Nutritional Biochemicals Corporation, Cleveland, Ohio) a crude enzyme preparation from hog pancreas, was used for the hydrolysis experiments. The digestion mixture contained the following substances in the amounts indicated:

fat sample	2.0 g
pancreatic lipase	500 mg
bile salts (Oxo Ltd., London, England)	15 mg
4% aqueous solution of CaCl_2	2 ml
distilled water	60 ml

Hydrolysis was carried out in a jacketed glass vessel under carefully controlled conditions of temperature, pH, and agitation. The temperature was maintained at 37 C by the circulation of water from a thermostat bath through the jacket, and the pH was maintained between 7.9 and 8.1 by means of a pH controller (Fermentation Design Inc., Edison, N.J.) which regulated the addition of 0.5N NaOH. The mixture was agitated violently throughout the hydrolysis period by a high speed mechanical stirrer.

The hydrolysis procedure was as follows:

(a) 60 ml of distilled water were poured into the reaction cell and allowed to warm to 37 C with stirring

(b) CaCl_2 solution and bile salts were added to the water

(c) the pH controller was turned on

(d) pancreatic lipase was added

The addition of pancreatic lipase lowered the pH of the mixture considerably and thus it was necessary to turn on the pH controller before the addition of the enzyme.

(e) fat was added

To ensure a quantitative transfer, the fat was weighed in a small container made of parafilm, and this "boat" and its contents were transferred to the reaction cell. The extent of hydrolysis could be ascertained by the volume of 0.5N NaOH added. Hydrolysis was continued until approximately 15% of the ester bonds of the fat sample were hydrolyzed, as calculated from the saponification value of the sample.

(f) 10 ml of 95% ethanol was added to stop the reaction

(g) after 30 seconds the stirrer was turned off, and the reaction mixture was poured into a 500-ml round bottom flask and dried on a rotary evaporator under vacuum at 70 C.

D. Extraction of Hydrolyzed Lipids

The lipids were extracted with two 200 ml volumes of diethyl ether: light petroleum ether (50% v/v). The ether solution was filtered, washed with five 50 ml volumes of 1% NaHCO_3 solution, dried over sodium sulfate, and the ether evaporated on a steam bath. It is important that the ether solution is washed completely free of pancreatic lipase and bile salts; if this is not done, the separation of the lipid classes by column chromatography will not be successful.

E. Separation of Glycerides Using Column Chromatography

The separation of mono-, di- and triglycerides was carried out using a method similar to that of Carroll (1961). The Florisil was deactivated with deionized water (7% w/v), and 12 g of this deactivated Florisil was packed in chromatographic columns of 1.3 cm diameter and 22 cm length. The samples were dissolved in hexane to a concentration of 100 mg per ml, and 1 ml was added, by pipette, directly to the column. Since monoglycerides are not readily soluble in hexane, the mixture was shaken before 1 ml was removed. This method was preferred

because direct application of glycerides to the column involved the tedious weighing of individual samples for each column.

Triglycerides were eluted with 100 ml of diethyl ether in hexane, (15% v/v), diglycerides with 100 ml of diethyl ether in hexane, (50% v/v), and monoglycerides with 100 ml of methanol in ether, (3% v/v). The eluates were evaporated and the residue weighed.

F. Characterization of Lipid Materials

Separation of the lipid classes by thin-layer chromatography on silica gel G plates (Privett et al., 1961) was used to check the purity of the fractions eluted from the Florisil columns.

The silica gel G slurry was made by mixing 1 part of silica gel G with 3 parts of distilled water. Twenty grams of silica gel G were sufficient to make five, 20 cm x 20 cm plates with a layer thickness of 0.25 mm. The plates were dried overnight at 120 C under vacuum and stored in a sealed cabinet containing a desiccant. The plates were activated for one hour under the same conditions immediately before use.

The lipid fractions were applied to the thin-layer plates as 10 μ liters of a 1% solution. The developing solvent was light petroleum ether: diethyl ether: acetic acid (90:10:1) and was allowed to travel to within 3.0 cm of the top of the plate for best separation; this took approximately 45 min. The spots were made visible with iodine vapour (Sims and Larose, 1962).

G. Preparation of Methyl Esters and Subsequent Analysis by Gas-Liquid Chromatography

Methyl esters of the component fatty acids were made according to the method of de Man (1964). The sealed bulbs were usually left over-night at 60 C to complete interesterification.

Difficulty was encountered when esterification of the feed lipids was attempted; the presence of free fatty acids in the samples retarded and often prevented methylation. To overcome this, the free fatty acids were neutralized with 0.1 N KOH in methanol using neutral litmus to indicate the end point. The samples were dark green in colour and so an indicator, such as phenolphthalein, could not be used. The neutral lipids were evaporated to dryness at 60 C under vacuum, and then esterified in exactly the same manner as

the porcine lipids.

Because of the difficulty encountered in esterifying the feed lipids, as mentioned above, two other esterification procedures were tried: one using boron trifluoride (Morrison and Smith, 1964) and one using concentrated HCl and methanol (Stoffel et al., 1959). Both of these methods were effective in esterifying the two ration samples.

Gas-liquid chromatographic analysis was carried out on an F. & M. (F. & M. Scientific Corp. Avondale, Pa.) Model 720 dual column, temperature programmed, gas chromatograph with a thermal conductivity detector. The 10ft x $\frac{1}{4}$ in OD stainless steel column was packed with 20% diethylene glycol succinate on firebrick, 60/80 mesh. Samples containing methyl esters of fatty acids with less than fourteen carbon atoms were injected at 80 C, programmed at 7.5 degrees per minute to 230 C, and held at that temperature until the last known peak was recorded. Those samples having methyl myristate (C_{14}) as the shortest chain, were chromatographed isothermally at 230 C. The injection temperature was 275 C, detector 240 C, and the flow rate of helium gas, 50 ml per minute in all cases.

The areas under the peaks were measured by a planimeter and converted to weight percentage methyl esters by using correction factors, established with pure compounds under

identical conditions (de Man, 1964). The correction factors for 20:0, 20:1, 20:2 and 22:0 were established by extrapolation of the curve plotted from the previously established correction factors (Figure 1).

H. Saponification Values

Saponification values were determined on representative samples following the method outlined by Mehlenbacher (1960). Two to 2.5 g of sample were used in the determinations.

I. Statistical Analysis

The data concerning fatty acid composition were subjected to analysis of variance (Steel and Torrie, 1960). Since the fatty acid composition was based on a total of 100%, the data were not orthogonal; thus an analysis of variance had to be carried out on each fatty acid. The data were normalized by an arc sin transformation before the statistical analysis was carried out. This was accomplished by reading the transformed values from Table 8.10, page 448 of the reference cited above. When data, necessary for the analysis, were missing, values were calculated according to the method outlined in section 8.5, page 139 of the cited reference. The multiple range test of Duncan (Steel and Torrie) was used to determine

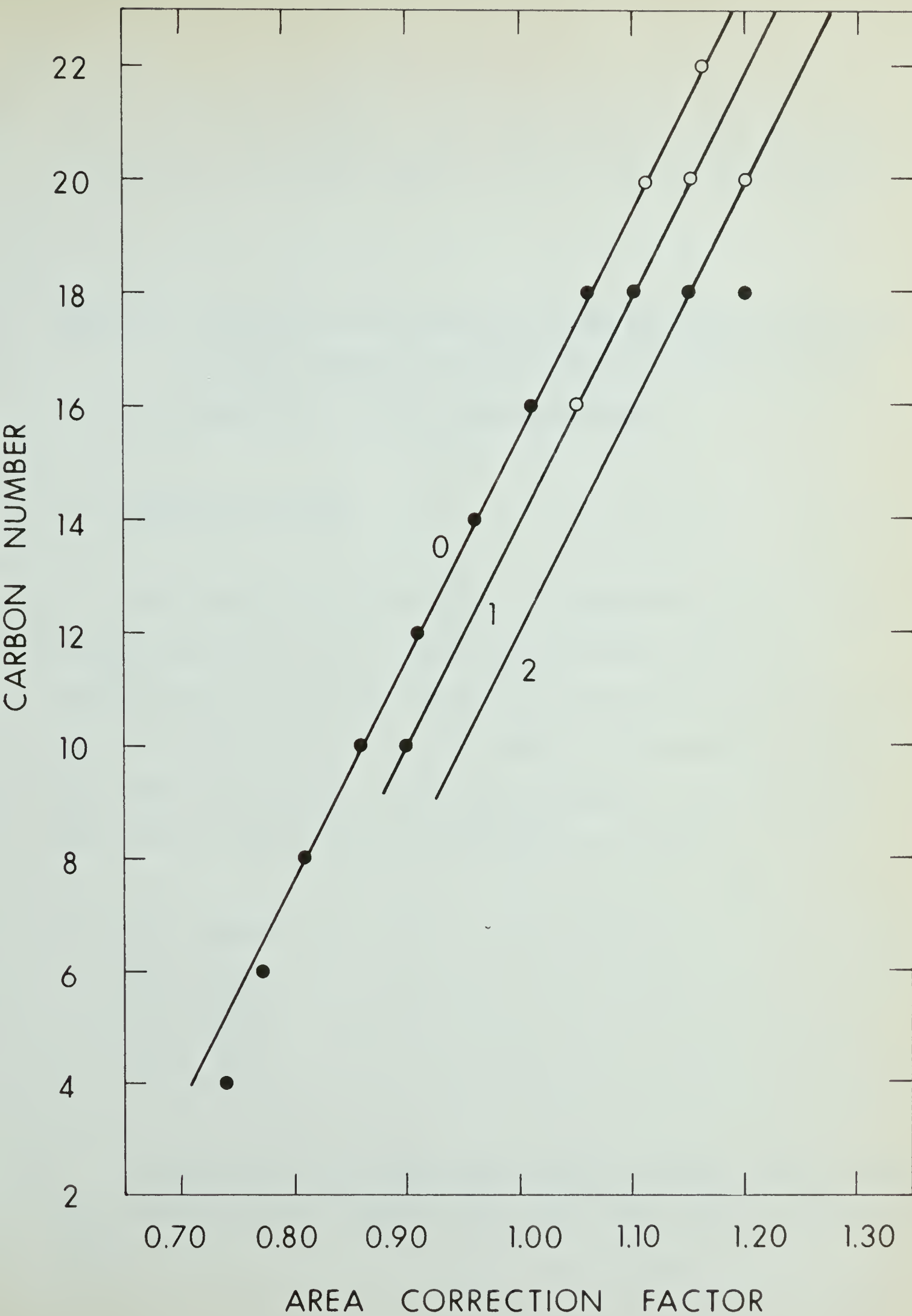


Figure 1. Methyl ester correction factors for fatty acids with 0, 1 and 2 double bonds (● from de Man, 1964, ○ extrapolated).

which of the fat samples were significantly different from each other, and which samples did not differ significantly.

PART II The Fatty Acid Composition and Glyceride Structure
of Lipid Samples from Piglets on a High Fat Diet;
Comparison of Five Sampling Sites

A. Source of Samples

Four piglets, ($\frac{3}{4}$ Yorkshire, $\frac{1}{4}$ Hampshire), eight weeks of age, previously on standard diets, were fed a diet containing 20% stabilized tallow. The other ingredients in the diet were essentially the same as the standard diet and are found in Table 1. The piglet samples, two from males and two from females, are designated as below:

OX - female

8 - female

3 - male

21 - male

At the end of an eight week feeding period, the piglets were killed and fat samples were taken from the following five sites:

- 1) Outer Shoulder
- 2) Inner Shoulder
- 3) Outer Loin
- 4) Inner Loin
- 5) Kidney

B. Methods

The procedures followed in this experiment were exactly the same as those described in Part I for the sow's body fat samples. A structural investigation of the fat from each site was carried out on two of the four replicates; these were chosen at random.

Part III Identification of Arachidic Acid

The chromatograms obtained after the gas-liquid chromatographic analysis of the lipid samples resembled those of Iverson et al. (1963) in that a peak with a retention time between the methyl esters of linoleic acid (octadecadienoic acid) and gadoleic acid (eicosenoic acid) was found (Figure 2).

The methods described below were carried out to determine whether peak 4 on the chromatogram in Figure 2, represented linolenic acid, arachidic acid or both of these acids.

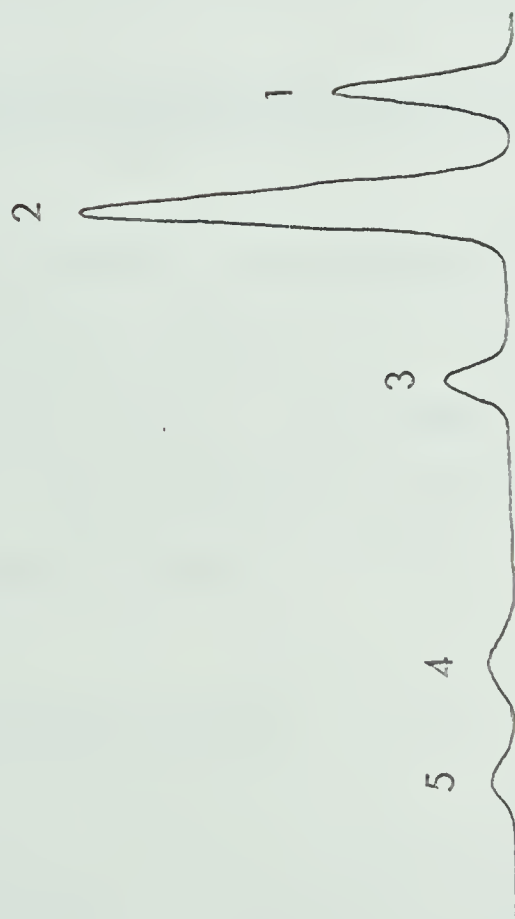


Figure 2. Portion of a gas chromatogram of methyl esters of piglet's kidney fat. Peaks in order of appearance: (1) 18:0, (2) 18:1, (3) 18:2, (4) 20:0, (5) 20:1.

A. Semilogarithmic Plots

The relative retention times of the component methyl esters versus number of carbon atoms, and the relative retention times of the component methyl esters versus number of double bonds were plotted on semilogarithm paper producing a straight line for members of a homologous series.

B. Gas-Liquid Chromatography of Standard Samples

Standard methyl linolenate and standard methyl eicosanoate (Applied Science Laboratories, Inc.) were chromatographed under the same conditions as described previously (Part I G.). The peaks on the chromatograms were identified by comparison with these reference esters.

C. Linolenic Acid Test

The presence of linolenic acid was tested using both the method described by Mehlenbacher (1960), and that of White and Brown (1949). The test was carried out on a representative sample from each source of fat studied, including the lipids extracted from the rations.

D. Separation of Methyl Ester by Gas Chromatography Using a Silicone Column

The diethylene glycol succinate column described earlier, separates the component methyl esters according to carbon number and unsaturation. A nonpolar silicone column, on the other hand, separates component methyl esters on the basis of their carbon number, and not according to their degree of unsaturation. Therefore, if the unknown peak were linolenic acid, it would have the same retention time as stearic, oleic and linoleic acids, all having eighteen carbon atoms; if the unknown peak were arachidic acid, it would be eluted at the same time as gadoleic acid, both having twenty carbon atoms.

An Aerograph Model A90P3 (Wilkins Instrument and Research Inc.) single column, thermal conductivity gas chromatograph was used to carry out the separation. The 5 ft x $\frac{1}{4}$ in OD stainless steel column was packed with 5% SE 30 on Chromsorb W 60/80 mesh. The column temperature was 220 C, the injector 240 C and detector 235 C. The flow rate of helium gas was 60 ml per minute.

To determine whether the fatty acid represented by the unknown peak contained eighteen or twenty carbon atoms, a

comparison was made of ratios of the C_{18} peak and C_{20} peak obtained by using the nonpolar silicone column and the polar diethylene glycol succinate column. Two ratios were established from the polar column: (1) a ratio designated as 3:2, in which stearate, oleate and linoleate are grouped and compared to the unknown peak plus gadoleate; this ratio assumes the unknown peak represents arachidic acid. (2) a ratio designated as 4:1 in which stearate, oleate, linoleate and the unknown peak are grouped and compared to gadoleate; this ratio assumes that the unknown peak represents linolenic acid.

The two ratios derived from the chromatograms of the polar column were compared to the ratio established by the use of the nonpolar column. Conclusions as to whether the unknown peak represents linolenic acid or arachidic acid were based upon which of the two ratios from the polar column, 3:2 or 4:1, was similar to the ratio obtained with nonpolar column.

RESULTS

Part I The Fatty Acid Composition and Glyceride Structure of Sow's Colostrum, Milk and Body Fat

A. Lipid Content of Colostrum and Milk Samples

Calculations based on the dry weight of samples, showed that sow's colostrum contained approximately 17% lipid, and sow's milk, taken from the first and third weeks of lactation, contained 32% and 31% lipid respectively.

B. Saponification Values of Colostrum, Milk, Body Tissue and Feed Lipids

The saponification values of the lipids studied in Part I are listed in Table 2.

C. Mono-, Di- and Triglyceride Content of the Hydrolyzed Lipid Samples

Results showed that when 15% of the ester bonds in the fat samples were hydrolyzed, a mixture of approximately 30% triglycerides, 45% diglycerides and 25% monoglycerides was obtained. These values represented only 60% of the total weight of lipid

Table 2. Saponification values of sow's colostrum fat, milk fat from the first and third weeks of lactation, body fat, and the lipids obtained from sow's gestation and lactation rations.

Fat sample	Saponification value ^a
Sow colostrum	189
milk 1st week	193
milk 3rd week	194
body fat	192
Gestation ration	180
Lactation ration	181

^a Saponification value is the number of mg of KOH required to saponify 1 g of fat.

applied to the column.

To determine the recovery of the three glyceride fractions from the Florisil columns, samples containing known amounts of these glycerides were reapplied to the columns. Total recovery was obtained when pure fractions were separated, which indicated that no loss of mono-, di- and triglycerides occurred during their separation.

D. Characterization of Lipid Materials by Thin-Layer Chromatography

Thin-layer chromatography (Figure 3) provided evidence that the mono-, di- and triglyceride fractions obtained after column chromatography were pure.

E. Fatty Acid Composition of Sow's Colostrum, Milk and Body Tissue Lipids

The fatty acid composition of colostrum, milk and depot fat from the four sows studied, are given in Table 3. Gas-liquid chromatograms of the methyl esters of sow's colostrum fat, milk fat and body fat are given in Figures 4, 5 and 6 respectively.

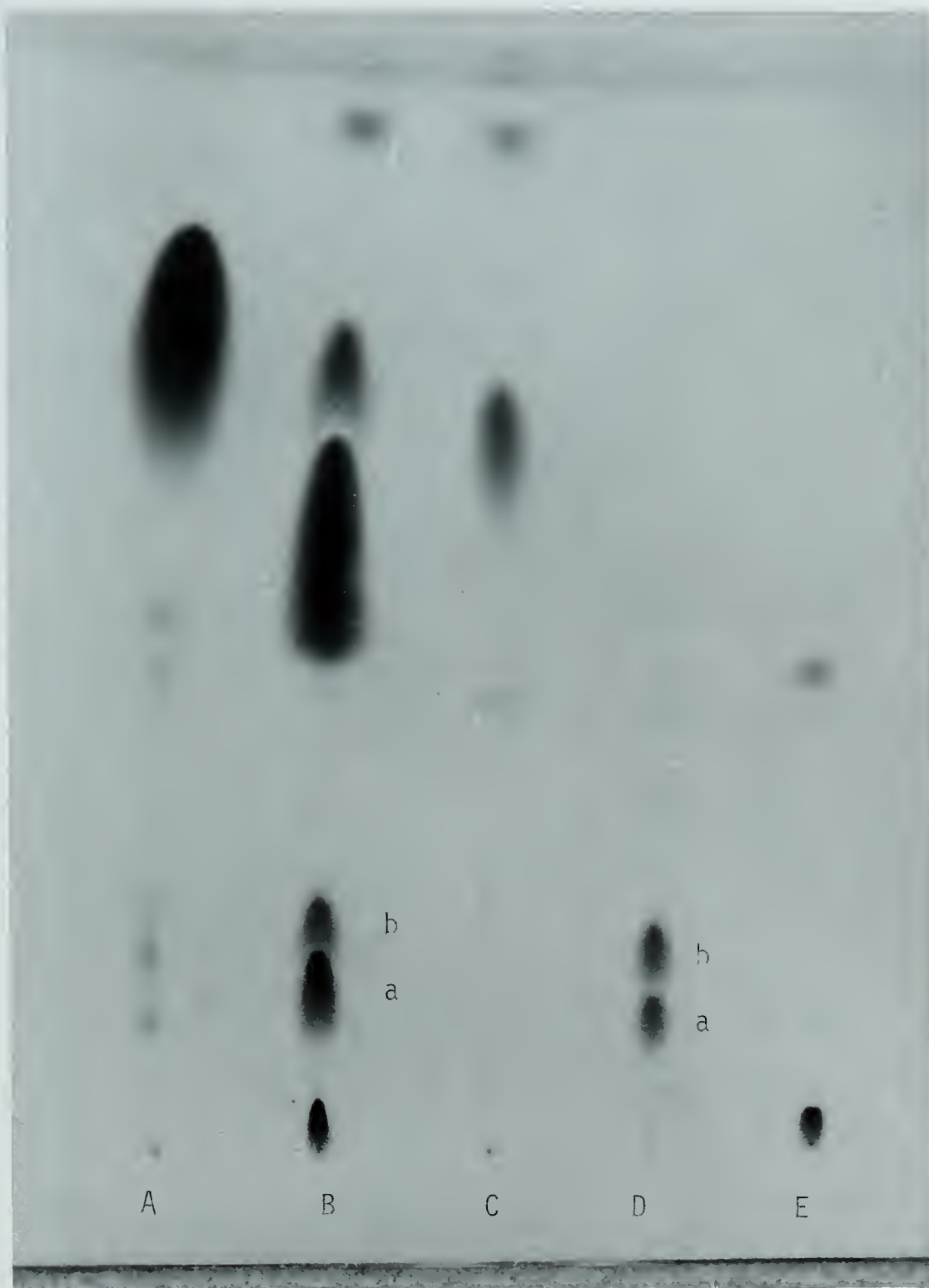


Figure 3. Thin-layer chromatogram of porcine body fat before and after hydrolysis. Samples: A. Porcine body fat before hydrolysis; B. Porcine body fat after hydrolysis; C. Triglyceride fraction of hydrolyzed body fat obtained by column chromatography on Florisil; D. Diglyceride fraction (separated into 1, 2 diglycerides (a) and 1, 3 diglycerides (b)); E. Monoglyceride fraction. Sample size: 10 microliters of a 1% solution in ether. Mobile phase, stationary phase and spot detection as described on P. 43.

Table 3. Fatty acid composition (weight %) of colostrum fat, body fat and milk fat from the first and third weeks of lactation, of sow's represented by replicates: 230X, 50, 230XX and 20.

Fatty acid ^a	Colostrum				Body Fat			
	230X	50	230XX	20	230X	50	230XX	20
10:0	- ^b	-	-	-	-	-	-	-
12:0	-	-	-	-	-	-	-	-
14:0	2.5	1.7	1.5	1.6	1.5	1.0	1.0	1.2
A	0.2	0.2	0.2	0.1	-	-	-	-
16:0	26.4	22.8	20.3	21.8	23.9	21.2	18.7	21.2
16:1	3.6	5.0	4.3	4.5	1.6	2.1	2.2	2.2
B	0.3	0.2	0.4	0.4	0.2	0.2	0.4	0.6
18:0	4.4	4.9	4.0	4.5	16.4	13.0	10.7	11.1
18:1	31.2	39.4	34.5	36.8	38.5	44.2	47.2	44.2
18:2	29.2	23.0	31.0	26.5	14.0	15.0	15.1	16.0
20:0	3.2	2.5	3.7	3.0	1.9	1.4	2.0	2.0
20:1	tr ^c	0.4	0.3	0.6	1.4	1.6	1.4	0.8
20:2	-	-	-	-	0.6	0.2	1.0	0.4
22:0	-	-	-	-	tr	tr	0.4	tr

a. Number of carbon atoms: number of double bonds
b. A dash indicates that the methyl ester was not detected on the gas chromatogram
c. tr indicates that a trace amount (<0.2% except for acids with retention times between 10:0 and A) of the methyl ester was detected on the gas chromatograms.

Table 3. (Continued)

Fatty acid	Milk 1st week			Milk 3rd week		
	230X	50	230XX	230X	50	230XX
10:0	0.1	0.2	0.2	0.2	0.2	0.2
12:0	0.3	0.2	0.4	0.4	0.3	0.3
14:0	3.8	3.6	4.1	4.8	4.2	4.3
A	0.2	0.6	0.2	0.4	0.4	0.6
16:0	30.8	29.2	32.2	34.4	31.8	34.9
16:1	12.8	12.9	10.4	13.4	12.8	13.8
B	tr	0.4	0.1	0.4	0.6	0.6
18:0	4.0	3.1	4.2	2.6	3.2	3.2
18:1	31.0	32.0	33.4	28.7	33.0	28.6
18:2	14.6	15.2	13.8	11.9	12.0	11.6
20:0	2.1	2.2	1.1	1.8	1.4	1.2
20:1	tr	0.2	tr	0.8	0.2	0.4
20:2	-	-	-	-	-	-
22:0	-	-	-	-	-	-

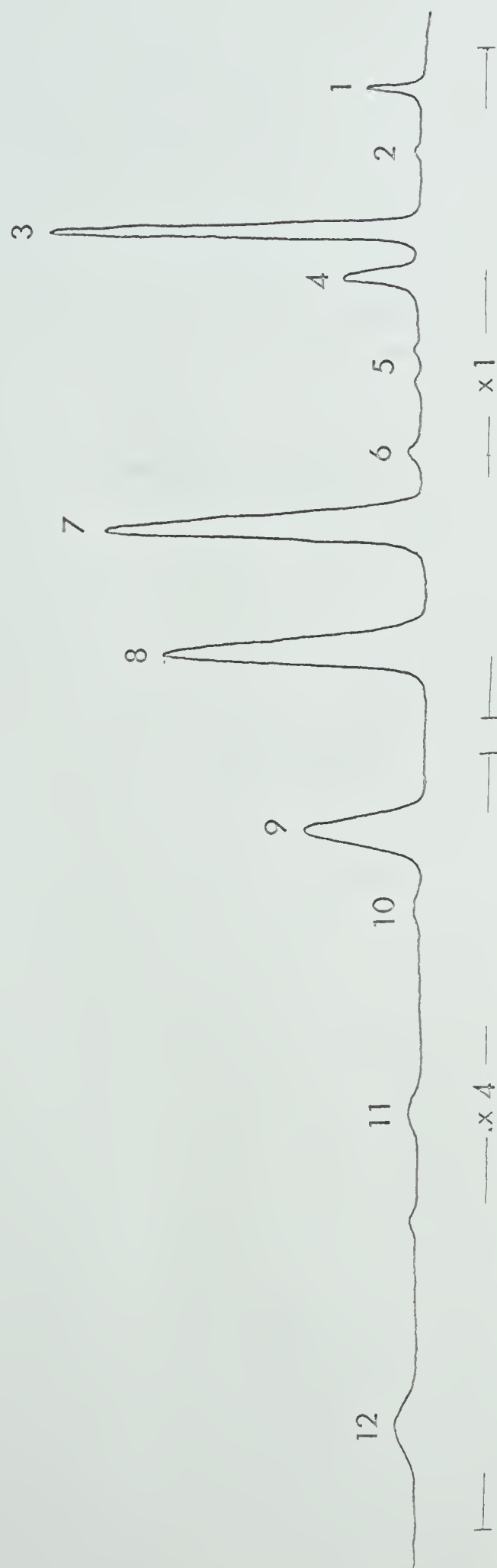


Figure 4. Gas chromatogram of methyl esters of sow's colostrum fat. Peaks in order of appearance: (1) 14:0, (2) 16:0, (3) 16:0, (4) 16:1, (5) 18:0, (6) 18:1, (7) 18:2, (8) 20:0, (9) 20:1, (10) 20:1, (11) 20:2, (12) 22:0. The peak between (11) and (12) was not identified.



Figure 5. Gas chromatogram of methyl esters of sow's milk fat. Peaks in order of appearance: (1) 10:0, (2) 12:0, (3) 14:0, (4) Δ , (5) 16:0, (6) 16:1, (7) 6, (8) 18:0, (9) 18:1, (10) 18:2, (11) 20:0, (12) 20:1.

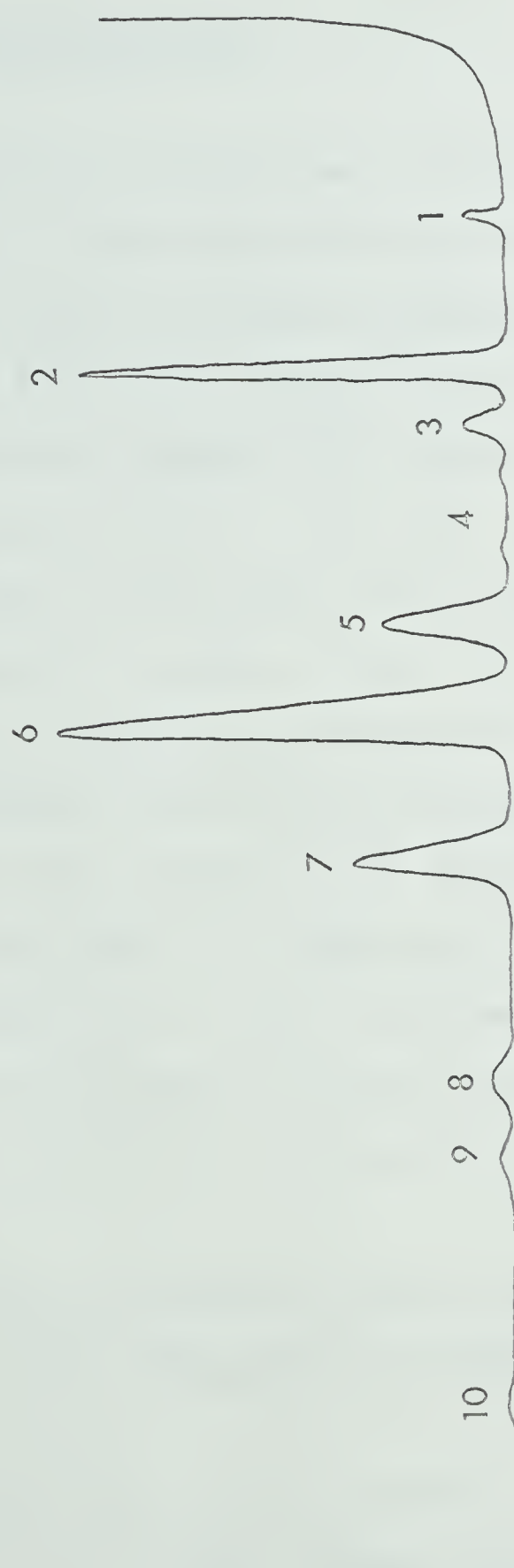


Figure 6. Gas chromatogram of methyl esters of sow's body fat. Peaks in order of appearance: (1) 14:0, (2) 16:0, (3) 16:1, (4) 6, (5) 18:0, (6) 18:1, (7) 18:2, (8) 20:0, (9) 20:1, (10) 20:2.

F. Statistical Analysis

Analyses of variance were carried out on the fatty acids of the porcine lipid samples to determine whether fat samples were significantly different from each other. The results showed that F was significant at the 1% level for the following acids: palmitic, palmitoleic, stearic, oleic, linoleic, arachidic and gadoleic. F was not significant at the 1% level for capric and lauric acids and the groups of unknown fatty acids denoted as A and B. Capric and lauric acids were present only in the milk fat samples, and the unknown fatty acids of groups A and B could be considered unimportant, as their total contribution to the fatty acid composition of a sample is less than 1%. Because of their insignificance, and the error involved in their measurement, groups A and B were omitted from the analysis using Duncan's new multiple range test. The results of this test have been recorded in Table 4.

G. Preparation of Methyl Esters of Feed Lipids Using Various Methods and Subsequent Gas-Liquid Chromatographic Analysis.

The feed lipids were esterified using the methods outlined by Stoffel et al. (1959), deMan (1964) and Morrison and Smith (1964). The results obtained following gas-liquid chromatography of the methyl esters showed that all of the methods of preparation gave similar results. The fatty acid composition

Table 4. Results of Duncan's new multiple range test (Steel and Tory, 1960) on the mean fatty acid composition (weight %) of sow's colostrum, milk and body fats. Means with the same superscript across the rows do not vary significantly ($P = 0.01$).

Fatty acid	Mean fatty acid composition			
	Colostrum	Body Fat	Milk 1st week	Milk 3rd week
14:0	1.8 ^{ab}	1.2 ^a	3.9 ^b	4.2 ^b
16:0	22.8 ^a	21.2 ^a	30.8 ^b	32.3 ^b
16:1	4.3 ^a	2.0 ^a	11.4 ^b	11.8 ^b
18:0	4.5 ^b	12.9 ^a	3.8 ^b	3.2 ^b
18:1	35.4 ^b	43.6 ^a	32.7 ^b	32.3 ^b
18:2	27.3 ^b	15.0 ^a	14.3 ^a	12.3 ^a
20:0	3.1 ^b	1.8 ^a	1.7 ^a	1.5 ^a
20:1	0.5 ^a	1.3 ^b	0.3 ^a	0.5 ^a

of the lipids obtained from the gestation and lactation rations, recorded in Table 5, are those found from the preparation of the methyl esters by the method of de Man.

H. Results of Data Obtained after Pancreatic Hydrolysis of Colostrum, Milk and Body Tissue Lipids

The fatty acid composition of the colostrum samples before hydrolysis and the composition of the purified mon-, di- and triglycerides obtained after 15% hydrolysis with pancreatic lipase is listed in Table 6. Similar data for the body fat samples and for the milk samples from the first and third weeks of lactation are given in Tables 7, 8 and 9. The four replicates of each type of sample were averaged and the results listed in Table 10.

The percentage of each component fatty acid in the 2-position of the glycerides studied can be obtained from the fatty acid composition of the original triglycerides and the 2-monoglycerides. The weight percent of a particular fatty acid esterified at the 2-position, according to Mattson and Volpenhein (1961a), will be equal to:

$$\frac{\% \text{ of the particular fatty acid in the monoglyceride formed}}{\% \text{ of the particular fatty acid in the original triglyceride} \times 3} \times 100.$$

Table 5. Fatty acid composition (weight %) of the lipids obtained from sow's gestation and lactation rations.

Fatty acid	Gestation ration	Lactation ration
14:0	0.6	1.1
16:0	18.9	19.8
16:1	1.0	0.9
18:0	3.5	6.1
18:1	32.9	30.2
18:2	34.6	35.3
18:3 & 20:0	8.0	5.3
20:1	0.5	0.9

Table 6. Fatty acid composition (weight %) of sow's colostrum fat before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 230X, 50, 230XX and 20 represent the four replicates.

Fatty acid	230X				50			
	Original ^a		TG ^b		Original		TG	
			DG ^b	MG ^b			DG	MG
10:0	-	-	-	-	-	-	0.9	9.0
12:0	-	-	-	-	-	-	-	-
14:0	2.5	2.4	2.9	4.3	1.7	1.7	2.6	5.7
A	0.2	0.3	tr	tr	0.2	tr	0.6	tr
16:0	26.4	23.4	30.8	43.8	22.8	24.3	28.6	40.4
16:1	3.6	4.1	3.6	4.8	5.0	4.7	5.2	6.2
B	0.3	0.4	tr	-	0.2	tr	tr	-
18:0	4.4	4.7	3.3	1.1	4.9	5.2	3.9	0.8
18:1	31.2	31.2	27.2	17.3	39.4	39.2	33.5	16.8
18:2	29.2	29.9	28.7	26.2	23.0	22.2	22.0	18.0
20:0	3.2	3.0	3.4	2.2	2.5	2.2	2.2	2.9
20:1	tr	0.3	-	-	0.4	0.4	0.2	-

- a. Original fatty acid composition before hydrolysis
- b. Fatty acid composition of partial glycerides obtained after hydrolysis, TG, DG and MG means tri-, di- and monoglycerides respectively.

Table 6. (Continued)

Fatty acid	230XX				20			
	Original		TG		Original		TG	
			DG	MG			DG	MG
10:0	-	tr	-	5.5	-	-	-	-
12:0	-	-	-	-	-	-	-	-
14:0	1.5	2.0	2.5	5.9	1.6	1.8	2.2	3.7
A	0.2	0.2	0.4	0.5	0.1	-	-	tr
16:0	20.3	17.9	40.2	48.3	21.8	15.0	33.2	59.8
16:1	4.3	4.9	5.6	5.1	4.5	6.2	5.2	3.6
B	0.4	0.5	tr	-	0.4	-	-	tr
18:0	4.0	1.2	1.3	0.9	4.5	0.9	1.9	1.7
18:1	34.5	35.9	30.4	14.3	36.8	44.2	35.7	14.7
18:2	31.0	33.0	18.5	15.7	26.5	28.3	20.5	14.7
20:0	3.7	4.1	0.9	3.5	3.0	3.4	1.1	1.7
20:1	0.3	0.2	tr	-	0.6	tr	-	tr

Table 7. Fatty acid composition (weight %) of sow's body fat before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 230X, 50, 230XX and 20 represent the four replicates.

Fatty acid	230X				50			
	Original		TG	DG	Original		TG	DG
			MG				MG	
10:0	-	tr	-	tr	-	0.7	-	tr
12:0	-	-	tr	tr	-	tr	-	-
14:0	1.5	1.7	2.6	5.6	1.0	1.6	1.5	3.8
A	-	-	-	-	-	-	-	-
16:0	23.9	25.6	48.0	66.8	21.2	22.9	39.7	63.5
16:1	1.6	1.7	1.9	2.5	2.1	2.7	2.8	3.3
B	0.2	-	-	-	0.2	tr	tr	tr
18:0	16.4	12.9	14.8	5.0	13.0	8.3	8.2	4.6
18:1	38.5	40.4	27.1	12.1	44.2	44.2	35.0	16.2
18:2	14.0	14.1	4.4	6.2	15.0	15.5	10.6	6.9
20:0	1.9	2.2	1.0	1.7	1.4	2.1	1.2	1.4
20:1	1.4	1.1	tr	tr	1.6	1.8	0.9	tr
20:2	0.6	-	-	-	0.2	-	-	-
22:0	tr	-	-	-	tr	-	-	-

Table 7. (Continued)

Fatty acid	230XX				20					
	Original		TG	DG	MG	Original		TG	DG	MG
10:0	-		0.9	-	-	-	-	-	-	-
12:0	-		-	-	-	-	-	-	-	-
14:0	1.0		1.0	1.4	4.1	1.2	1.3	2.1	3.3	
A	-		-	-	-	-	-	-	-	-
16:0	18.7		17.8	31.1	61.8	21.2	21.2	36.5	64.7	
16:1	2.2		2.6	2.4	3.3	2.2	2.2	2.8	3.0	
B	0.4		-	-	-	0.6	-	-	-	-
18:0	10.7		5.2	5.5	3.5	11.1	3.8	3.8	3.5	
18:1	47.2		51.2	45.1	18.0	44.2	48.8	42.6	15.0	
18:2	15.1		17.1	11.4	6.6	16.0	18.9	9.7	7.2	
20:0	2.0		2.3	1.6	2.0	2.0	2.7	1.0	2.5	
20:1	1.4		1.5	1.2	0.5	0.8	1.0	1.2	0.5	
20:2	1.0		-	-	-	0.4	-	-	-	-
22:0	0.4		-	-	-	tr	-	-	-	-

Table 8. Fatty acid composition (weight %) of sow's milk fat from the first week of lactation before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 230X, 50, 230XX and 20 represent the four replicates.

Fatty acid	230X				50			
	Original		TG		Original		TG	
			DG	MG			DG	MG
10:0	0.1	0.6	1.2	12.1	0.2	0.5	-	-
12:0	0.3	0.6	0.6	1.6	0.2	0.3	tr	-
14:0	3.8	4.7	5.3	5.5	3.6	3.7	4.9	6.5
A	0.2	0.6	0.8	1.3	0.6	0.6	-	-
16:0	30.8	31.3	37.7	33.2	29.2	18.9	38.3	65.4
16:1	12.8	12.3	13.9	10.2	12.9	18.6	15.6	10.3
B	-	-	-	-	0.4	-	-	-
18:0	4.0	2.2	1.3	2.0	3.1	0.9	0.9	0.4
18:1	31.0	30.5	25.5	17.9	32.0	38.1	27.5	9.2
18:2	14.6	13.8	11.5	6.9	15.2	16.3	11.1	7.1
20:0	2.1	2.3	1.6	7.9	2.2	2.0	1.1	1.0
20:1	tr	0.8	0.3	1.1	0.2	tr	tr	tr

Table 8. (Continued)

Fatty acid	230XX				20			
	Original	TG	DG	MG	Original	TG	DG	MG
10:0	0.2	0.8	0.5	5.2	0.2	0.4	0.5	3.1
12:0	0.4	0.4	0.4	0.9	0.2	0.4	0.7	-
14:0	4.1	4.4	5.3	6.5	4.2	4.1	4.2	5.8
A	0.2	0.5	0.6	-	0.6	-	-	-
16:0	32.2	33.1	45.0	49.9	31.2	27.7	38.5	46.1
16:1	10.4	14.4	12.7	11.2	9.7	8.1	7.7	7.9
B	0.1	-	-	-	0.5	-	-	-
18:0	4.2	2.9	2.1	1.3	3.9	3.5	2.9	2.6
18:1	33.4	28.8	22.1	13.4	34.4	38.5	30.3	23.6
18:2	13.8	12.4	9.6	6.9	13.4	14.4	13.4	9.0
20:0	1.1	1.6	1.4	3.6	1.4	2.0	1.6	1.7
20:1	tr	0.5	tr	0.9	0.4	0.5	tr	tr

Table 9. Fatty acid composition (weight %) of sow's milk fat from the third week of lactation before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 230X, 50, 230XX and 20 represent the four replicates.

Fatty acid	230X				50			
	Original		TG		Original		TG	
			DG	MG			DG	MG
10:0	0.2	0.2	tr	0.9	0.2	0.1	0.1	0.3
12:0	0.4	0.4	0.3	0.7	0.3	0.1	0.3	0.5
14:0	4.8	4.2	5.0	6.2	4.2	3.7	4.6	5.7
A	0.4	-	tr	0.5	0.4	-	-	0.4
16:0	34.0	31.7	37.1	43.5	31.8	28.5	37.5	40.0
16:1	13.4	12.1	13.7	15.7	12.8	12.8	14.0	17.1
B	0.4	-	tr	0.7	0.6	-	-	0.6
18:0	2.6	4.3	3.3	1.4	3.2	3.5	2.4	0.7
18:1	28.7	30.8	25.5	15.7	33.0	33.0	27.5	18.6
18:2	11.9	14.8	13.4	12.6	12.0	15.7	13.1	14.1
20:0	1.8	1.2	1.5	1.8	1.4	2.2	0.2	1.8
20:1	0.8	-	-	-	0.2	-	-	-

Table 9. (Continued)

Fatty acid	230XX				20			
	Original		TG	DG	Original		TG	DG
			MG				MG	
10:0	0.2	1.3	0.1	0.4	-	0.2	-	-
12:0	0.3	0.3	0.2	0.3	0.4	0.2	0.5	0.4
14:0	4.3	4.4	3.9	5.4	3.7	3.9	3.9	6.8
A	0.6	0.2	tr	0.5	0.4	0.5	0.4	0.8
16:0	34.9	27.1	35.4	38.7	28.0	34.4	30.0	40.1
16:1	13.8	8.6	9.7	15.1	7.9	10.7	9.9	12.7
B	0.6	tr	tr	0.4	0.8	1.1	0.3	0.4
18:0	3.2	5.1	3.6	0.9	3.9	3.6	3.9	1.1
18:1	28.6	35.9	30.0	21.8	39.2	31.4	34.7	22.6
18:2	11.6	15.3	15.3	14.5	13.6	12.6	14.7	13.3
20:0	1.2	1.5	1.6	1.7	1.6	1.2	1.4	1.5
20:1	0.4	-	-	-	0.6	-	-	-

Table 10. Fatty acid composition (weight %) of sow's colostrum, milk and body fats before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase; average of four replicates.

Fatty acid	Colostrum				Body Fat			
	TG		DG		TG		DG	
	Original	MG	Original	MG	Original	MG	Original	MG
10:0	-	-	-	3.6	-	-	-	-
12:0	-	-	-	-	-	-	-	-
14:0	1.8	2.0	2.6	4.9	1.2	1.4	1.9	4.2
A	0.2	0.1	0.2	0.1	-	-	-	-
16:0	22.8	20.2	33.2	48.1	21.2	21.9	38.8	64.2
16:1	4.4	5.0	4.9	4.9	2.0	2.3	2.5	3.0
B	0.3	0.2	tr	-	0.4	tr	-	-
18:0	4.4	3.0	2.6	1.1	12.8	7.6	8.1	4.2
18:1	35.5	37.6	31.7	15.8	43.5	46.1	37.4	15.3
18:2	27.4	28.4	22.4	18.6	15.0	16.4	9.0	6.7
20:0	3.1	3.2	1.9	2.6	1.8	2.3	1.2	1.9
20:1	0.3	0.2	tr	-	1.3	1.4	0.8	0.2
20:2	-	-	-	-	0.6	0.1	-	-
22:0	-	-	-	-	-	-	-	-

Table 10. (Continued)

Fatty acid	Milk 1st week				Milk 3rd week			
	Original	TG	DG	MG	Original	TG	DG	MG
10:0	0.2	0.6	0.6	5.1	0.2	0.4	tr	0.4
12:0	0.3	0.4	0.4	0.6	0.4	0.2	0.4	0.5
14:0	3.9	4.2	4.9	6.1	4.2	4.0	4.4	6.0
A	0.4	0.4	0.4	0.3	0.4	0.3	0.1	0.6
16:0	30.8	27.8	40.0	48.6	32.2	30.4	35.0	40.6
16:1	11.4	10.8	12.5	9.9	12.0	11.4	11.8	15.2
B	-	-	-	-	0.6	0.3	0.1	0.5
18:0	3.8	2.4	1.8	1.6	3.2	4.1	3.3	1.0
18:1	32.7	34.0	26.4	16.0	32.4	32.8	26.4	19.7
18:2	14.2	14.2	11.4	7.5	12.3	14.6	13.8	13.6
20:0	1.7	2.0	1.4	3.6	1.5	1.5	1.2	1.7
20:1	0.1	0.4	tr	0.5	0.5	-	-	-
20:2	-	-	-	-	-	-	-	-
22:0	-	-	-	-	-	-	-	-

In Tables 11, 12, 13 and 14 are listed the percentage of each fatty acid in the 2-position of the glycerides of colostrum fat, body fat and milk fats from the first and third weeks of lactation. These data have been averaged, and the means are given in Table 15.

The glyceride structures of colostrum, milk and body fat were compared on the basis of percentage saturated fatty acids at the 2-position (Table 16). Fatty acids with chain lengths between fourteen and eighteen carbon atoms were included in the calculations. These were carried out by summing both the saturated fatty acid composition of the original fat and that of the 2-monoglycerides reported in Table 15. The percentage of saturated fatty acids at the 2-position was determined as outlined in the preceding paragraph.

Glyceride types were determined according to the method of Vander Wal (1960) and are given in Table 17. Determination of glyceride types was based on the average of four replicates from Table 10.

Table 11. Percentage of fatty acids esterified at the 2-position of
sow's colostrum fat, calculated according to the method of
Mattson and Volpenhein (1961a).

Fatty acid	230X	50	230XX	20
14:0	Original 2-monoglyceride % 2-position	2.5 4.3 57	1.7 5.7 112	1.6 3.7 77
16:0	Original 2-monoglyceride % 2-position	26.4 43.8 55	22.8 40.4 59	21.8 59.8 91
16:1	Original 2-monoglyceride % 2-position	3.6 4.8 44	5.0 6.2 41	4.5 3.6 27
18:0	Original 2-monoglycerides % 2-position	4.4 1.1 8	4.9 0.8 8	4.5 1.7 12
18:1	Original 2-monoglyceride % 2-position	31.2 17.3 18	39.4 16.8 14	36.8 14.7 13
18:2	Original 2-monoglyceride % 2-position	29.2 26.2 30	23.0 18.0 26	26.5 14.7 18
20:0	Original 2-monoglyceride % 2-position	3.2 2.2 23	2.5 2.9 39	3.0 1.7 19
20:1	Original 2-monoglyceride % 2-position	tr - <33	0.4 - <33	0.6 tr <33

Table 12. Percentage of fatty acids esterified at the 2-position of sow's body fat, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid	230X	50	230XX	20
14:0	Original 2-monoglyceride % 2-position	1.5 5.6 124	1.0 3.8 127	1.2 3.3 92
16:0	Original 2-monoglyceride % 2-position	23.9 66.8 93	21.2 63.5 100	21.2 64.7 102
16:1	Original 2-monoglyceride % 2-position	1.6 2.5 52	2.1 3.3 53	2.2 3.0 45
18:0	Original 2-monoglyceride % 2-position	16.4 5.0 10	13.0 4.6 12	11.1 3.5 10
18:1	Original 2-monoglyceride % 2-position	38.5 12.1 10	44.2 16.2 12	44.2 15.0 11
18:2	Original 2-monoglyceride % 2-position	14.0 6.2 15	15.0 6.9 15	16.0 7.2 15
20:0	Original 2-monoglyceride % 2-position	1.9 1.7 30	1.4 1.4 33	2.0 2.5 42
20:1	Original 2-monoglyceride % 2-position	1.4 tr <33	1.4 tr <33	0.8 0.5 21

Table 13. Percentage of fatty acids esterified at the 2-position of sow's milk fat from the first week of lactation, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid	230X	50	230XX	20
10:0	Original 2-monoglyceride % 2-position	0.1 12.1 4033	0.2 5.2 866	0.2 3.1 517
12:0	Original 2-monoglyceride % 2-position	0.3 1.6 178	0.4 0.9 75	0.2 - <33
14:0	Original 2-monoglyceride % 2-position	3.8 5.5 48	4.1 6.5 53	4.2 5.8 46
16:0	Original 2-monoglyceride % 2-position	30.8 33.2 36	32.2 49.9 52	31.2 46.1 49
16:1	Original 2-monoglyceride % 2-position	12.8 10.2 26	10.4 11.2 36	9.7 7.9 27
18:0	Original 2-monoglyceride % 2-position	4.0 2.0 17	4.2 1.3 10	3.9 2.6 22
18:1	Original 2-monoglyceride % 2-position	31.0 17.9 19	33.4 13.4 13	34.4 23.6 23
18:2	Original 2-monoglyceride % 2-position	14.6 6.9 16	13.8 6.9 17	13.4 9.0 22
20:0	Original 2-monoglyceride % 2-position	2.1 7.9 125	1.1 3.6 109	1.4 1.7 40
20:1	Original 2-monoglyceride % 2-position	tr 1.1 >33	tr 0.9 >33	0.4 tr <33

Table 14. Percentage of fatty acids esterified at the 2-position of sow's milk fat from the third week of lactation, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid	230X	50	230XX	20
10:0	Original 2-monoglyceride % 2-position	0.2 0.9 150	0.2 0.4 67	- - -
12:0	Original 2-monoglyceride % 2-position	0.4 0.7 58	0.3 0.3 33	0.4 0.4 33
14:0	Original 2-monoglyceride % 2-position	4.8 6.2 43	4.3 5.4 42	3.7 6.8 61
16:0	Original 2-monoglyceride % 2-position	34.0 43.5 43	34.9 38.7 37	28.0 40.1 48
16:1	Original 2-monoglyceride % 2-position	13.4 15.7 39	13.8 15.1 36	7.9 12.7 54
18:0	Original 2-monoglyceride % 2-position	2.6 1.4 18	3.2 0.9 9	3.9 1.1 9
18:1	Original 2-monoglyceride % 2-position	28.7 15.7 18	28.6 21.8 25	39.2 22.6 19
18:2	Original 2-monoglyceride % 2-position	11.9 12.6 35	11.6 14.5 42	13.6 13.3 32
20:0	Original 2-monoglyceride % 2-position	1.8 1.8 33	1.2 1.7 47	1.6 1.5 31
20:1	Original 2-monoglyceride % 2-position	0.8 - <33	0.4 - <33	0.6 - <33

Table 15. Percentage of fatty acids esterified at the 2-position of sow's colostrum, milk and body fats, calculated according to the method of Mattson and Volpenhein (1961a); average of four replicates.

Fatty acid	Colostrum	Milk 1st week	Milk 3rd week	Body fat
10:0	Original 2-monoglyceride % 2-position	0.2 5.1 850	0.2 0.4 67	- - -
12:0	Original 2-monoglyceride % 2-position	0.3 0.6 67	0.4 0.5 42	- - -
14:0	Original 2-monoglyceride % 2-position	3.9 6.1 52	4.2 6.0 48	1.2 4.2 117
16:0	Original 2-monoglyceride % 2-position	22.8 48.1 70	32.2 40.6 42	21.2 64.2 101
16:1	Original 2-monoglyceride % 2-position	4.4 4.9 37	12.0 15.2 42	2.0 3.0 50
18:0	Original 2-monoglyceride % 2-position	4.4 1.1 8	3.2 1.0 10	12.8 4.2 11
18:1	Original 2-monoglyceride % 2-position	35.5 15.8 15	32.4 19.7 20	43.5 15.3 12
18:2	Original 2-monoglyceride % 2-position	27.4 18.6 23	12.3 13.6 37	15.0 6.7 15
20:0	Original 2-monoglyceride % 2-position	3.1 2.6 28	1.5 1.7 38	1.8 1.9 35
20:1	Original 2-monoglyceride % 2-position	0.3 - <33	0.5 - <33	1.3 0.2 5

Table 16. A comparison of colostrum, milk and body fats on a basis of the percentage saturated fatty acids at the 2-position. The basis of the calculations is given on page 77.

Fat sample	Original	2-monoglyceride	% at 2-position
Colostrum	29.0	54.1	62
Milk 1st week	38.5	56.3	49
Milk 3rd week	39.6	47.6	40
Body Fat	35.2	72.6	69

Table 17. Glyceride types calculated from pancreatic hydrolysis data according to Vander Wal (1960). Calculations are based on the average of four replicates for each source of fat (Table 10).

Fat sample	Composition: Types (weight %)				Composition: Isomers (weight %)			
	GS ₃	GS ₂ U	GSU ₂	GU ₃	SUS	SSU	USU	UUS
Colostrum	2.1	19.5	51.0	27.4	1.5	10.0	38.2	12.8
Milk 1st week	5.2	29.3	47.7	17.8	2.8	26.5	33.8	13.9
Milk 3rd week	7.1	30.5	42.9	19.5	7.0	23.5	19.5	23.3
Body fat	2.4	23.2	57.3	17.0	0.8	22.4	49.7	7.6

I. The Preferential Incorporation of Fatty Acids at the 2-Position of Porcine Glycerides

Comparisons were made between short and long chain fatty acids at the 2-position, and between saturated and unsaturated fatty acids at the 2-position, (Table 18) to determine whether the shorter chain fatty acids or the saturated fatty acids have a greater preference for the internal position on porcine triglyceride molecules. Myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids were used in the comparisons, and the percentages at the 2-position were calculated by averaging the component percentages listed in Table 15.

J. Results of Data Obtained After Pancreatic Hydrolysis of the Feed Lipids

The fatty acid composition of the lipids before hydrolysis and of the purified mono-, di- and triglycerides after 15% hydrolysis with pancreatic lipase are given in Table 19.

Table 18. Proportions of fatty acids at the 2-positions in the glycerides of colostrum, milk and body fats, based on unsaturation and chain. The basis for the calculations is given on page 85.

Fat sample	Proportion at the 2-position based on unsaturation		Proportion at the 2-position based on chain length	
	<u>saturated^a</u>	<u>unsaturated^b</u>	<u>shorter chains^c</u>	<u>longer chains^d</u>
Colostrum	56	25	66	15
Milk 1st week	40	21	45	16
Milk 3rd week	33	33	44	22
Body fat	76	26	89	13

- a. Saturated fatty acids included are: 14:0, 16:0 and 18:0
b. Unsaturated fatty acids included are: 16:1, 18:1 and 18:2
c. Shorter chain fatty acids included are: 14:0, 16:0 and 16:1
d. Longer chain fatty acids included are: 18:0, 18:1 and 18:2

Table 19. Fatty acid composition (weight %) of lipids obtained from sow's gestation and lactation rations before hydrolysis, and the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase.

Fatty acid	Gestation ration			Lactation ration		
	Original	TG	DG	Original	TG	DG
14:0	0.6	0.6	0.8	1.1	1.2	1.4
16:0	18.9	13.5	13.3	19.8	15.5	15.0
16:1	1.0	1.3	0.6	0.9	1.6	0.9
18:0	3.5	1.8	2.1	6.1	2.8	2.8
18:1	32.9	36.5	36.7	30.2	35.4	35.1
18:2	34.6	41.0	42.0	35.3	38.7	39.8
18:3 + 20:00	8.0	5.0	3.8	5.3	4.6	4.5
20:1	0.5	tr	0.5	0.9	tr	0.4
			-			-

Part II The Fatty Acid Composition and Glyceride Structure
of Lipid Samples from Piglets on a High Fat Diet;
Comparison of Five Sampling Sites

A. Saponification Values of the Body Tissue Lipids of Piglets

The saponification values of the fat samples from the piglets on a high fat diet, containing 20% stabilized tallow, are given in Table 20.

B. Mono-, Di- and Triglyceride Content of the Hydrolyzed
Lipid Samples

The amounts of mono-, di- and triglycerides recovered after column chromatography on Florisil were similar to those recovered in Part I.

C. Fatty Acid Composition of Body Tissue Lipids from Various
Sites

The fatty acid composition of lipids obtained from the outer and inner shoulder, outer and inner loin, and kidney are given in Table 21. A gas-liquid chromatogram of the methyl esters of piglet's kidney fat is illustrated in Figure 7.

Table 20. Saponification of Piglet Body Tissue Fat.

Sample site	Saponification value
Outer shoulder	194
Inner shoulder	187
Outer loin	191
Inner loin	189
Kidney	196

Table 21. Fatty acid composition (weight %) of tissue fat from the outer and inner shoulder, outer and inner loin, and kidney of piglets on a high fat diet represented by replicates 8, 21, 3 and 0X.

Fatty acid	Outer loin				Inner loin				Kidney			
	8	21	3	0X	8	21	3	0X	8	21	3	0X
14:0	1.6	1.7	1.6	1.8	1.4	2.0	1.7	2.0	1.8	2.2	2.0	2.2
16:0	21.7	21.6	21.7	21.5	21.6	22.7	22.4	21.7	24.4	26.1	24.6	24.5
16:1	3.7	4.2	3.5	3.8	2.9	3.7	4.0	3.5	2.8	3.4	3.0	2.9
B	1.2	1.1	1.4	1.4	1.4	1.2	1.3	1.3	1.4	1.1	1.4	1.4
18:0	9.4	8.5	10.0	8.8	10.7	11.4	11.3	10.8	14.9	13.4	14.3	13.2
18:1	53.9	54.7	53.8	54.3	53.5	50.2	50.5	52.1	45.8	45.7	46.0	46.7
18:2	5.6	6.1	6.4	6.0	6.2	6.8	6.7	6.4	7.0	6.4	6.9	6.9
20:0	1.0	0.8	0.7	0.8	0.9	0.9	1.0	1.0	0.9	0.7	0.8	0.9
20:1	1.7	1.1	0.6	1.2	1.1	0.8	0.8	1.0	0.8	0.8	0.8	1.1

Table 21. (Continued)

Fatty acid	Outer shoulder				Inner shoulder			
	8	21	3	0X	8	21	3	0X
14:0	2.0	1.9	1.6	1.7	1.7	1.6	1.9	1.8
16:0	22.1	22.1	21.4	21.4	22.1	22.7	22.5	21.8
16:1	4.2	4.2	4.4	4.2	3.5	3.9	3.7	3.4
B	1.5	1.4	1.4	1.6	1.4	1.2	1.5	1.4
18:0	7.9	8.8	10.2	8.8	11.7	10.7	12.1	11.1
18:1	54.2	52.6	52.8	54.0	51.0	51.4	48.9	51.6
18:2	6.7	6.8	6.4	6.5	6.7	6.8	7.3	7.0
20:0	0.7	1.0	0.8	0.8	0.9	0.7	1.0	0.8
20:1	0.4	1.1	0.8	0.8	0.8	0.7	0.9	0.8

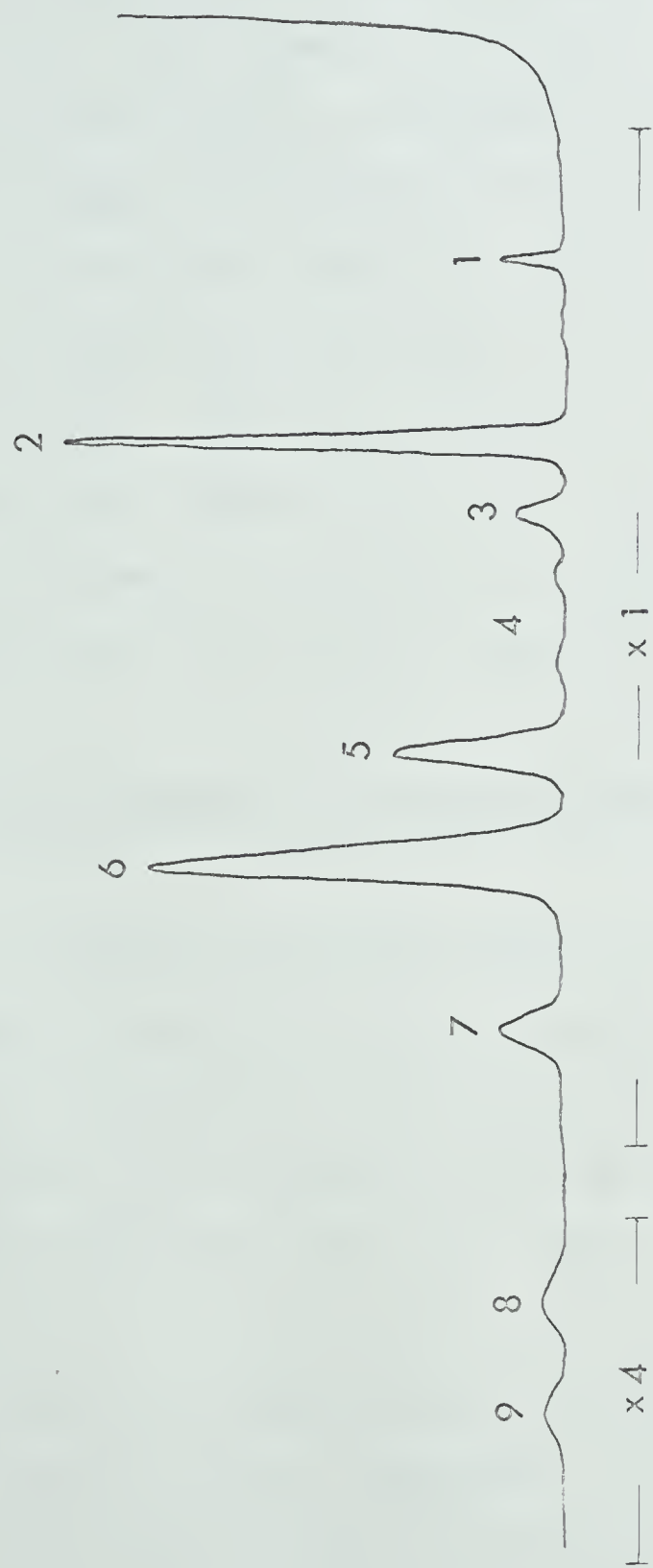


Figure 7. Gas chromatogram of methyl esters of piglet's kidney fat. Peaks in order of appearance: (1) 14:0, (2) 16:0, (3) 16:1, (4) 6, (5) 13:0, (6) 13:1, (7) 13:2, (8) 20:0, (9) 20:1.

D. Statistical Analysis

Analyses of variance were carried out on the fatty acids of the porcine lipid samples to determine whether sampling sites were significantly different from each other. F was found to be significant at the 1% level for the following acids: palmitic, palmitoleic, stearic, oleic and linoleic. F was not found to be significant at either the 1% or 5% level in the following acids: myristic, arachidic, gadoleic and the group of unknown acids referred to as B. As in Part I, group B was omitted from Duncan's new multiple range test. The results of Duncan's test are given in Table 22.

E. Results of Hydrolysis of Body Tissue Lipids

Two of the four replicate samples from each site were hydrolyzed, and replicates were chosen completely at random. Because no difference in fatty acid composition was noted between sexes, no consideration was given to the sex of the piglet from which the sample was taken.

The fatty acid composition of the original outer shoulder fat, and that of the purified mono-, di- and triglycerides obtained after 15% hydrolysis with pancreatic lipase is given in Table 23. Likewise, the fatty acid composition of fats

Table 22. Results of Duncan's new multiple range test on the mean fatty acid composition of piglet glycerides obtained from various sites. Means with the same superscript across the rows do not vary significantly ($P=0.01$).

Fatty acid	Outer shoulder	Inner shoulder	Outer loin	Inner loin	Kidney
14:0	1.8 ^a	1.8 ^a	1.7 ^a	1.8 ^a	2.0 ^a
16:0	21.8 ^a	22.3 ^a	21.6 ^a	22.3 ^a	24.9 ^b
16:1	4.2 ^c	3.6 ^b	3.8 ^{bc}	3.5 ^{ab}	3.0 ^a
18:0	8.9 ^a	11.4 ^b	9.2 ^a	11.1 ^b	13.9 ^c
18:1	53.4 ^c	50.7 ^b	54.2 ^c	51.6 ^b	46.1 ^a
18:2	6.6 ^{ab}	6.9 ^b	6.0 ^a	6.5 ^{ab}	6.8 ^b
20:0	0.8 ^a	0.8 ^a	0.8 ^a	0.9 ^a	0.8 ^a
20:1	0.8 ^a	0.8 ^a	1.1 ^a	0.9 ^a	0.9 ^a

Table 23. Fatty acid composition (weight %) of piglet's outer shoulder fat before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 21 and 3 represent the two replicates.

	21				3			
	Original	TG	DG	MG	Original	TG	DG	MG
14:0	1.9	1.5	2.2	4.4	1.6	1.5	2.0	4.3
16:0	22.1	21.4	33.5	63.3	21.4	20.9	33.7	62.5
16:1	4.2	4.1	5.1	6.4	4.4	3.7	3.7	6.5
B	1.4	tr	tr	tr	1.4	tr	tr	tr
18:0	8.8	6.3	7.6	3.2	10.2	5.9	5.6	3.3
18:1	52.6	57.1	44.2	20.0	52.8	59.1	47.7	20.4
18:2	6.8	7.2	5.5	2.0	6.4	7.2	5.7	2.4
20:0	1.0	1.2	0.8	0.6	0.8	1.0	0.8	0.5
20:1	1.1	1.1	0.8	-	0.8	0.6	0.6	-

from the other sites, before and after hydrolysis, are given in Tables 24, 25, 26 and 27.

The percentages of fatty acids esterified at the 2-position of glycerides from the various sites examined, were calculated as outlined in Part I, p.65, and are listed in the following tables: Table 28, outer shoulder; Table 29, inner shoulder; Table 30, outer loin; Table 31, inner loin; and Table 32, kidney. The results from each site were averaged and the mean percentages at the 2-position are given in Table 33.

The proportions of fatty acids at the 2-position in the piglet glycerides, based on unsaturation and chain length, were calculated by averaging the proportions of component fatty acids at the 2-position (from Table 33) as carried out in Part I, p.85. The results are given in Table 34.

Glyceride types (Table 35) were determined from the pancreatic hydrolysis data according to the method of Vander Wal (1960); calculations were carried out on the average of two replicates for each source of fat.

Table 24. Fatty acid composition (weight %) of piglet's inner shoulder fat before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 8 and 21 represent the two replicates.

	8				21			
	Original	TG	DG	MG	Original	TG	DG	MG
14:0	1.7	1.5	2.3	3.8	1.6	1.4	2.5	4.8
16:0	22.1	22.0	34.5	64.4	22.7	23.6	35.3	65.0
16:1	3.5	3.4	4.1	3.5	3.9	2.8	3.6	5.7
B	1.4	tr	tr	0.7	1.2	tr	tr	1.1
18:0	11.7	6.2	7.0	4.2	10.7	9.4	10.8	3.2
18:1	51.0	57.6	44.5	20.7	51.4	54.2	41.0	17.6
18:2	6.7	7.3	5.5	2.6	6.8	6.1	4.7	2.4
20:0	0.9	1.0	0.9	tr	0.7	1.0	1.1	0.4
20:1	0.8	0.8	1.1	tr	0.7	1.4	0.8	tr

Table 25. Fatty acid composition (weight %) of piglet's outer loin fat before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 3 and 0X represent the two replicates.

	3				0X			
	Original	TG	DG	MG	Original	TG	DG	MG
14:0	1.6	1.3	1.9	4.2	1.8	1.3	2.5	5.0
16:0	21.7	19.6	32.7	60.3	21.5	20.4	32.2	59.7
16:1	3.5	3.3	3.9	5.6	3.8	3.8	4.8	6.4
B	1.4	tr	tr	1.0	1.4	tr	tr	1.3
18:0	10.0	5.1	5.3	3.6	8.8	9.7	8.3	2.9
18:1	53.8	60.8	48.9	21.7	54.3	55.6	44.2	21.0
18:2	6.4	7.5	5.7	2.9	6.0	6.2	5.1	2.9
20:0	0.7	1.2	0.7	0.6	0.8	1.3	1.0	0.5
20:1	0.6	1.0	0.7	tr	1.2	1.4	1.1	tr

Table 26. Fatty acid composition (weight %) of piglet's inner loin fat before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 8 and 3 represent the two replicates.

	8				3			
	Original	TG	DG	MG	Original	TG	DG	MG
14:0	1.4	1.7	2.7	3.7	1.7	1.4	2.1	4.0
16:0	21.6	24.3	34.9	62.3	22.4	21.4	32.7	61.1
16:1	2.9	3.3	3.5	5.3	4.0	3.4	3.8	5.6
B	1.4	tr	0.5	0.7	1.3	tr	1.3	0.6
18:0	10.7	6.5	7.7	3.7	11.3	10.3	9.6	3.4
18:1	53.5	56.4	44.2	21.1	50.5	54.6	43.0	21.8
18:2	6.2	6.3	5.2	2.3	6.7	7.0	6.0	2.3
20:0	0.9	0.9	0.4	0.6	1.0	0.9	0.9	0.9
20:1	1.1	0.5	0.5	tr	0.8	0.9	0.5	tr

Table 27. Fatty acid composition (weight %) of piglet's kidney fat before hydrolysis and of the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase. 8 and 0X represent the two replicates.

	8				0X			
	Original	TG	DG	MG	Original	TG	DG	MG
14:0	1.8	2.1	2.7	3.4	2.2	2.4	3.4	3.6
16:0	24.4	25.2	41.0	66.2	24.5	22.7	39.8	71.8
16:1	2.8	3.4	3.2	2.2	2.9	4.5	3.7	1.9
B	1.4	tr	tr	tr	1.4	tr	tr	tr
18:0	14.9	2.8	5.6	3.6	13.2	2.9	6.6	4.3
18:1	45.8	56.2	40.4	21.6	46.7	55.1	39.1	16.3
18:2	7.0	9.0	5.4	2.7	6.9	10.3	5.5	2.0
20:0	0.9	0.8	1.1	tr	0.9	1.6	1.1	tr
20:1	0.8	0.3	0.4	-	1.1	0.3	0.5	-

Table 28. Percentage of fatty acids esterified at the 2-position of glycerides from the outer shoulder of piglets on a high fat diet, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid		21	3
14:0	Original	1.9	1.6
	2-monoglyceride	4.4	4.3
	% 2-position	77	90
16:0	Original	22.1	21.4
	2-monoglyceride	63.3	62.5
	% 2-position	95	97
16:1	Original	4.2	4.4
	2-monoglyceride	6.4	6.5
	% 2-position	51	49
18:0	Original	8.8	10.2
	2-monoglyceride	3.2	3.3
	% 2-position	12	11
18:1	Original	52.6	52.8
	2-monoglyceride	20.0	20.4
	% 2-position	13	13
18:2	Original	6.8	6.4
	2-monoglyceride	2.0	2.4
	% 2-position	10	12
20:0	Original	1.0	0.8
	2-monoglyceride	0.6	0.5
	% 2-position	20	21
20:1	Original	1.1	0.8
	2-monoglyceride	-	-
	% 2-position	<33	<33

Table 29. Percentage of fatty acids esterified at the 2-position of glycerides from the inner shoulder of piglets on a high fat diet, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid		8	21
14:0	Original	1.7	1.6
	2-monoglyceride	3.8	4.8
	% 2-position	74	100
16:0	Original	22.1	22.7
	2-monoglyceride	64.4	65.0
	% 2-position	97	95
16:1	Original	3.5	3.9
	2-monoglyceride	3.5	5.7
	% 2-position	33	49
18:0	Original	11.7	10.7
	2-monoglyceride	4.2	3.2
	% 2-position	12	10
18:1	Original	51.0	51.4
	2-monoglyceride	20.7	17.6
	% 2-position	14	11
18:2	Original	6.7	6.8
	2-monoglyceride	2.6	2.4
	% 2-position	13	12
20:0	Original	0.9	0.7
	2-monoglyceride	tr	0.4
	% 2-position	<33	19
20:1	Original	0.8	0.7
	2-monoglyceride	tr	tr
	% 2-position	<33	<33

Table 30. Percentage of fatty acids esterified at the 2-position of glycerides from the outer loin of piglets on a high fat diet, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid		3	0X
14:0	Original	1.6	1.8
	2-monoglyceride	4.2	5.0
	% 2-position	88	92
16:0	Original	21.7	21.5
	2-monoglyceride	60.3	59.7
	% 2-position	93	92
16:1	Original	3.5	3.8
	2-monoglyceride	5.6	6.4
	% 2-position	53	56
18:0	Original	10.0	8.8
	2-monoglyceride	3.6	2.9
	% 2-position	12	11
18:1	Original	53.8	54.3
	2-monoglyceride	21.7	21.0
	% 2-position	13	13
18:2	Original	6.4	6.0
	2-monoglyceride	2.9	2.9
	% 2-position	15	16
20:0	Original	0.7	0.8
	2-monoglyceride	0.6	0.5
	% 2-position	28	21
20:1	Original	0.6	1.2
	2-monoglyceride	tr	tr
	% 2-position	<33	<33

Table 31. Percentage of fatty acids esterified at the 2-position of glycerides from the inner loin of piglets on a high fat diet, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid		8	3
14:0	Original	1.4	1.7
	2-monoglyceride	3.7	4.0
	% 2-position	88	78
16:0	Original	21.6	22.4
	2-monoglyceride	62.3	61.1
	% 2-position	96	91
16:1	Original	2.9	4.0
	2-monoglyceride	5.3	5.6
	% 2-position	61	47
18:0	Original	10.7	11.3
	2-monoglyceride	3.7	3.4
	% 2-position	12	10
18:1	Original	53.5	50.5
	2-monoglyceride	21.1	21.8
	% 2-position	13	14
18:2	Original	6.2	6.7
	2-monoglyceride	2.3	2.3
	% 2-position	12	11
20:0	Original	0.9	1.0
	2-monoglyceride	0.6	0.9
	% 2-position	22	30
20:1	Original	1.1	0.8
	2-monoglyceride	tr	tr
	% 2-position	<33	<33

Table 32. Percentage of fatty acids esterified at the 2-position of glycerides from the kidney of piglets on a high fat diet, calculated according to the method of Mattson and Volpenhein (1961a).

Fatty acid		8	OX
14:0	Original	1.8	2.2
	2-monoglyceride	3.4	3.6
	% 2-position	63	54
16:0	Original	24.4	24.5
	2-monoglyceride	66.2	71.8
	% 2-position	90	98
16:1	Original	2.8	2.9
	2-monoglyceride	2.2	1.9
	% 2-position	26	22
18:0	Original	14.9	13.2
	2-monoglyceride	3.6	4.3
	% 2-position	8	11
18:1	Original	45.8	46.7
	2-monoglyceride	21.6	16.3
	% 2-position	16	12
18:2	Original	7.0	6.9
	2-monoglyceride	2.7	2.0
	% 2-position	13	10
20:0	Original	0.9	0.9
	2-monoglyceride	tr	tr
	% 2-position	<33	<33
20:1	Original	0.8	1.1
	2-monoglyceride	-	-
	% 2-position	<33	<33

Table 33. Percentage of fatty acids esterified at the 2-position of glycerides from the outer and inner shoulder, outer and inner loin, and kidney of piglets on a high fat diet, calculated according to the method of Mattson and Volpenhein (1961a); average of two replicates.

Fatty acid	Outer shoulder	Inner shoulder	Outer loin	Inner loin	Kidney
14:0	84	87	90	83	58
16:0	96	96	92	94	94
16:1	50	41	54	54	24
18:0	12	11	12	11	10
18:1	13	12	13	14	14
18:2	11	12	16	12	12
20:0	20	19	24	26	<33
20:1	<33	<33	<33	<33	<33

Table 34. Proportions of fatty acids at the 2-position in the glycerides of outer and inner shoulder, outer and inner loin, and kidney fats of piglets on a high fat diet. The basis of the calculations is given on page 95.

Sample site	Proportion at the 2-position based on unsaturation	Proportion at the 2-position based on chain length	
		saturated ^a	unsaturation ^b
Outer shoulder	64	25	77
Inner shoulder	65	22	75
Outer loin	65	28	79
Inner loin	63	27	77
Kidney	54	17	59

- a. Saturated fatty acids included are: 14:0, 16:0 and 18:0
b. Unsaturated fatty acids included are: 16:1, 18:1 and 18:2
c. Shorter chain fatty acids included are: 14:0, 16:0 and 16:1
d. Longer chain fatty acids included are: 18:0, 18:1 and 18:2

Table 35. Glyceride types calculated from pancreatic hydrolysis data according to Vander Wal (1960). Calculations are based on the average of two replicates for each source of fat.

Sample site	Composition: Types (weight %)			Composition: Isomers (weight %)			
	GS ₃	GS ₂ U	GSU ₂	GU ₃	SUS	SSU	USU UUS
Outer shoulder	1.4	17.8	59.0	21.7	0.6	17.2	51.8 7.2
Inner shoulder	2.1	20.8	56.1	21.1	0.9	19.8	47.2 8.9
Outer loin	1.6	18.2	57.1	23.2	0.7	17.4	48.8 8.3
Inner loin	2.0	20.7	58.2	19.0	0.7	20.0	50.7 7.5
Kidney	3.6	27.2	54.8	14.4	1.1	26.0	46.7 8.0

F. Fatty Acid Composition of Stabilized Tallow

The fatty acid composition of the tallow lipids used in the piglets' high fat diet, and the composition of the purified mono-, di- and triglycerides obtained after 15% hydrolysis with pancreatic lipase are given in Table 36.

Part III Identification of Arachidic Acid

A. Semilogarithmic Plots

The curves obtained by plotting the log of the retention time versus carbon number and the log of the retention time versus number of double bonds are given in Figures 8 and 9 respectively.

B. Gas-Liquid Chromatography of Standard Samples

Both standard methyl linoleate and standard methyl eicosanoate had the same retention time as that of the unknown peak.

C. Linolenic Acid Test

Hexabromide crystals, having a melting point between 170 C

Table 36. Fatty acid composition (weight %) of tallow lipids from the piglets' high fat diet before hydrolysis and the purified partial glycerides obtained after 15% hydrolysis with pancreatic lipase.

Fatty acid	Original	TG	DG	MG
14:0	2.7	3.0	3.7	7.0
A	0.9	0.7	1.0	2.3
16:0	25.2	16.5	25.7	36.6
16:1	3.6	4.9	4.4	5.0
B	1.2	0.5	tr	tr
18:0	18.4	7.9	9.7	7.3
18:1	43.3	59.3	51.7	37.4
18:2	3.2	5.6	2.9	2.6
20:0	0.7	0.8	0.6	1.2
20:1	0.5	0.7	0.3	0.4

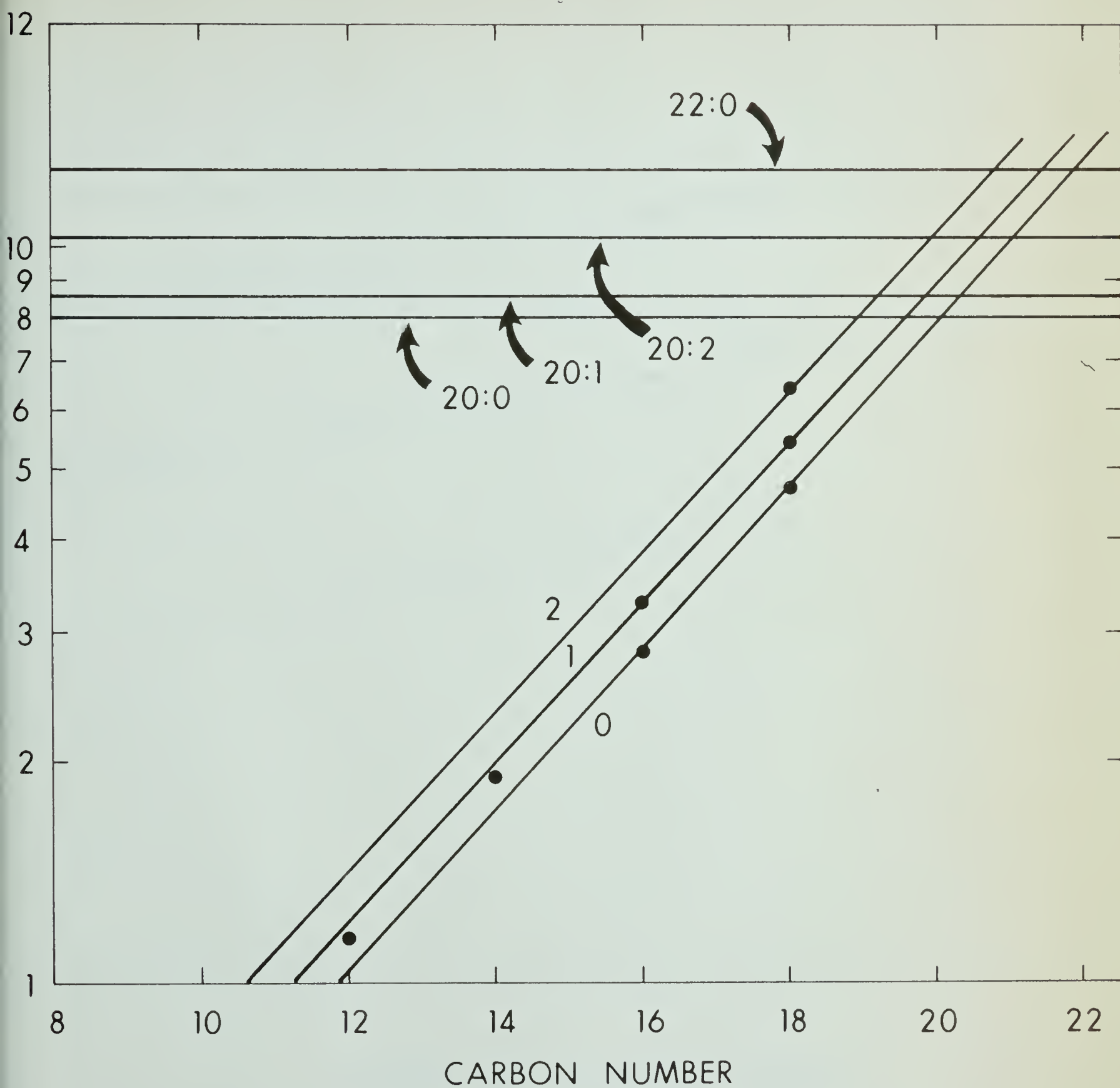


Figure 8. Semilogarithmic plot of retention time vs. carbon number for 0, 1 and 2 double bonds of methyl esters of porcine fats.

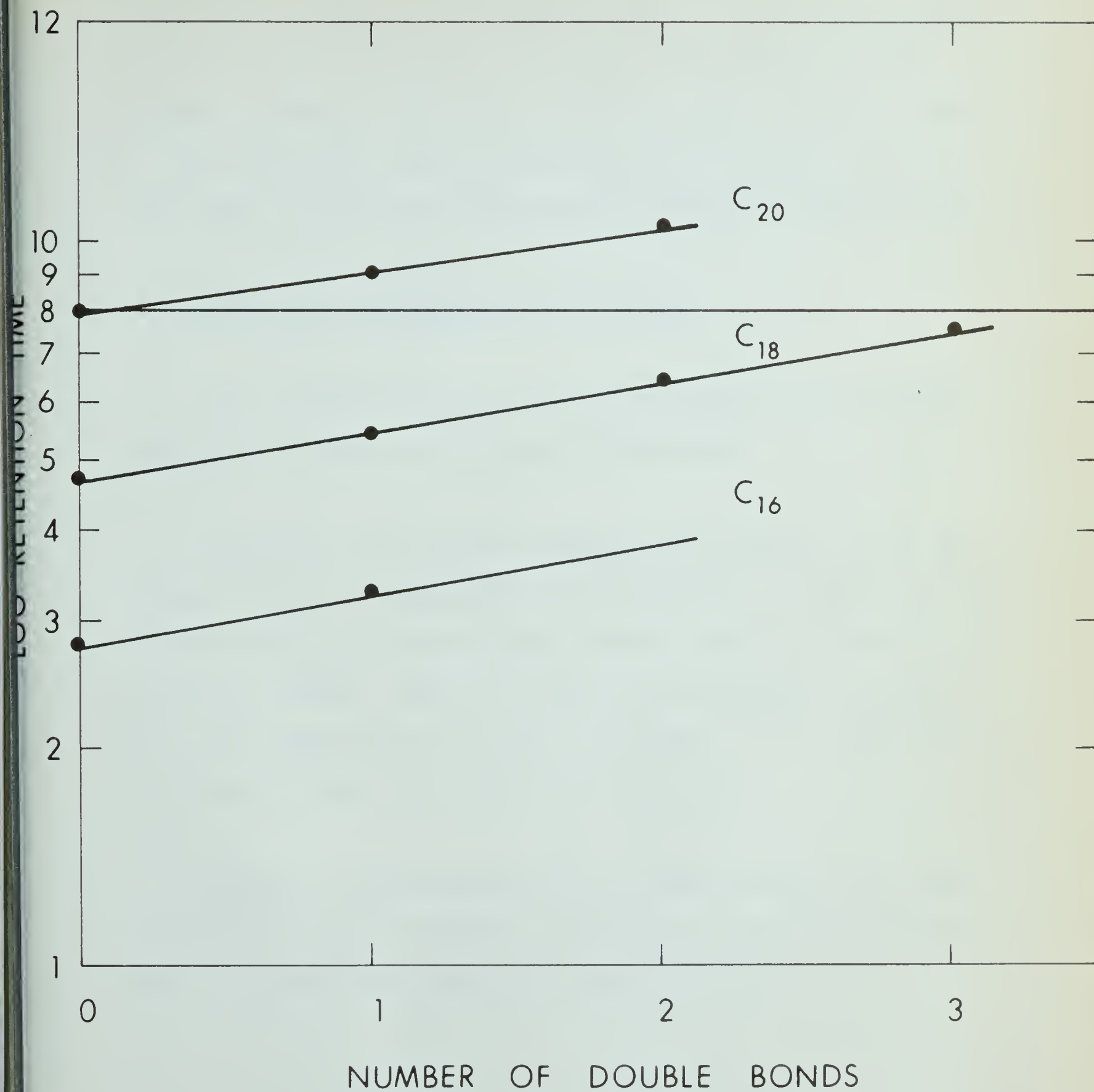


Figure 9. Semilogarithmic plot of retention time vs. number of double bonds for 16, 18 and 20 carbon atoms of methyl esters of porcine fats.

and 180 C, could not be obtained when the fat samples were tested. Thus a negative result was recorded with each sample studied. A positive result was noted, however, when a fat sample was tested containing pure linolenic acid to the level of detection specified by White and Brown (1949).

D. Gas-Liquid Chromatographic Analysis

The results of the analyses, in terms of the proposed peak area ratios are recorded in Table 37.

A portion of a typical chromatogram (Figure 2) of the methyl esters obtained with the diethylene glycol succinate column shows the unknown peak and the peaks included in the proposed ratios. Peaks 1, 2 and 3 are compared to peaks 4 and 5 to obtain the 3:2 ratio, and peaks 1, 2, 3 and 4 are compared to peak 5 to obtain the 4:1 ratio.

A typical chromatogram of the methyl esters with the silicone column represented in Figure 10, shows the peaks which were used to obtain the peak area ratio.

Table 37. Peak area ratios obtained from the gas-liquid chromatographic analysis of lipid samples on polar (DEGS- diethylene glycol succinate) and nonpolar (SE 30) columns. Refer to page 53 for the explanation of peak area ratios.

Fat sample	Peak area ratio 3:2 on DEGS		Peak area ratio 4:1 on DEGS		Peak area ratio C ₁₈ :C ₂₀ on SF 30	
Sow: colostrum	20:1		200:1		16:1	
milk 1st week	21:1		205:1		35:1	
milk 3rd week	26:1		102:1		28:1	
body fat	24:1		51:1		25:1	
Piglet: outer shoulder	44:1		89:1		34:1	
inner shoulder	41:1		83:1		38:1	
outer loin	35:1		56:1		36:1	
inner loin	36:1		72:1		36:1	
kidney	44:1		83:1		46:1	
Ration: gestation	10:1		122:1		50:1	
lactation	12:1		80:1		52:1	
stabilized tallow	54:1		127:1		58:1	

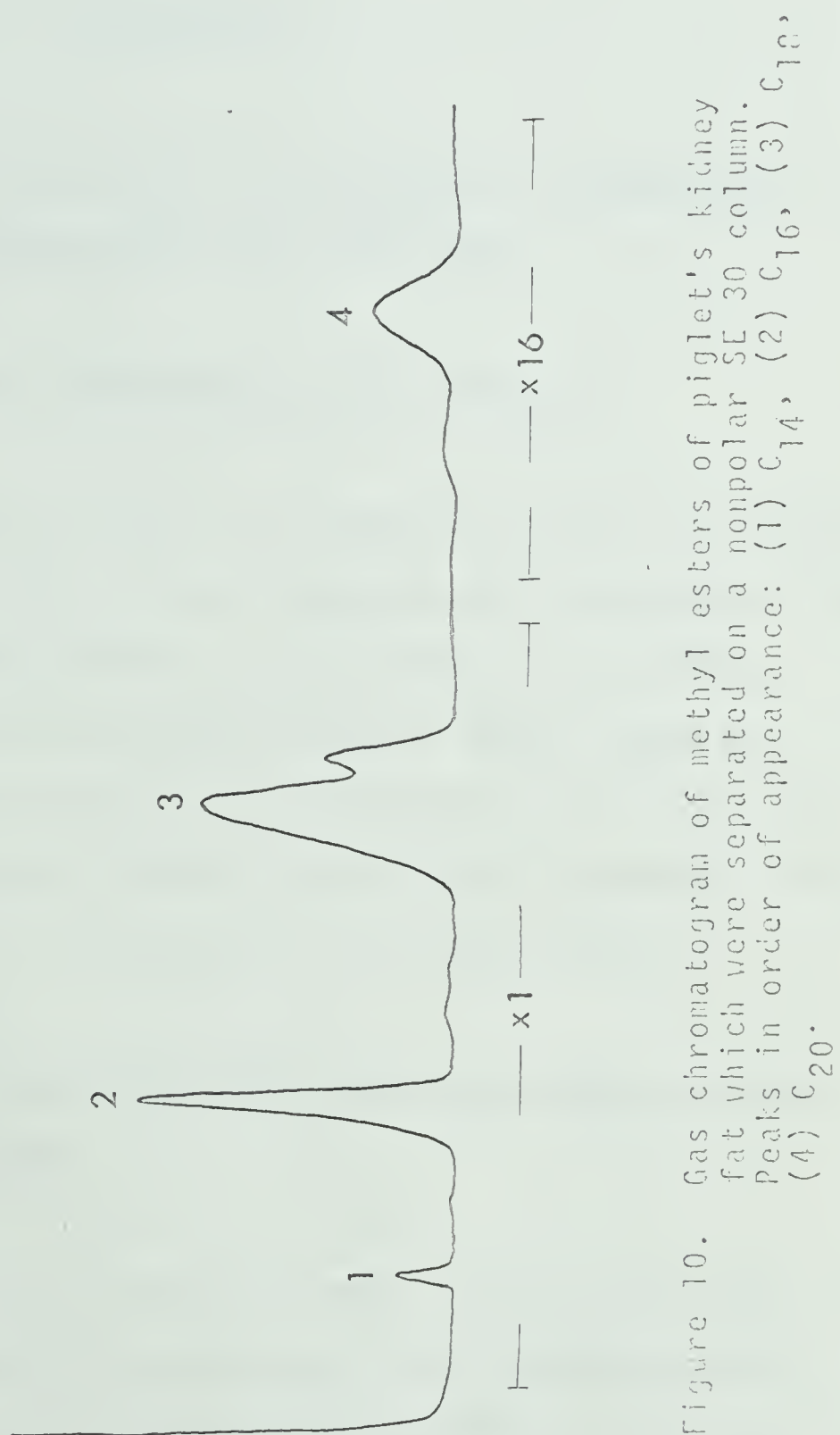


Figure 10. Gas chromatogram of methyl esters of piglet's kidney fat which were separated on a nonpolar SE 30 column. Peaks in order of appearance: (1) C_{14} , (2) C_{16} , (3) C_{18} , (4) C_{20} .

DISCUSSION

Part I Fatty Acid Composition and Glyceride Structure of Sow's Colostrum, Milk and Body Fat

A. Lipid Content of Colostrum and Milk Samples

The fat content of sow's colostrum was found to be lower than that of sow's milk. These findings are in agreement with those reported in the literature (Bowland et al., 1949a; Sheffy et al., 1952; de Man and Bowland, 1963), although direct comparison of the results obtained in this study with those of previous work is difficult, because previous results were not based on the dry weight of the milk samples.

B. Saponification Values of Sow's Colostrum, Milk and Body Fat and Feed Lipids

The saponification values of sow's colostrum and milk fat agree with those reported by Sheffy et al. (1952), and the saponification value of the depot fat is within the range for lard reported by Mehlenbacher (1960). The saponification values of the feed fats are lower than those of the porcine fats, which may reflect the difference in fatty acid composition. The saponification values were used to determine how many ester

bonds in the fat samples must be split to achieve 15% hydrolysis.

C. Fatty Acid Composition of Sow's Colostrum, Milk and Body Fat

Results obtained concerning the fatty acid composition of sow's colostrum, milk and body fat were similar to those of de Man and Bowland (1963). Colostrum and body fat contained no capric and lauric acids, while milk fat from the first and third weeks of lactation contained small amounts of both of these acids. Colostrum and body fat contained higher amounts of fatty acids with eighteen carbon atoms than did milk fat, colostrum having a strikingly high amount of linoleic acid, and body fat containing more stearic acid than the other porcine fats. The level of detection of the gas chromatograph used was only sufficient to pick up the higher levels of eicosadienoic and behenic acids.

An analysis of variance of the component fatty acids in the fat samples, and the application of Duncan's new multiple range test to these data (Table 4) brought out the major differences in composition between the samples investigated. There were no significant differences between the fatty acid composition of milk from the first week of lactation and that from the third week. Both milk fat

samples were significantly different from the body fat in myristic, palmitic and palmitoleic acids, and colostrum fat was significantly different from milk fat in palmitic and palmitoleic acids. Colostrum and body fat did not vary significantly in the shorter chain fatty acids (myristic, palmitic and palmitoleic), but there were significant differences between these two samples as far as long chain fatty acids were concerned. A study of the longer chain fatty acids (those with eighteen or twenty carbon atoms) revealed that, for any long chain fatty acid, either colostrum fat or body fat was similar to milk fat, but both colostrum and body fat were never similar to milk fat at the same time.

An interesting observation from Duncan's test was that the composition of milk fat from the third week of lactation was always at one extreme of the fatty acid means, and either colostrum or body fat at the other extreme of the fatty acid means. Although milk fat from the first week of lactation was, in many cases, significantly different from colostrum and body fat, this fat was nearer in composition to colostrum and body fat than was milk fat from the third week of lactation.

D. Preparation of the Methyl Esters of Feed Lipids Using Various Methods

The three methods of preparing methyl esters (Stoffel et al., 1959; de Man, 1964; Morrison and Smith, 1964) gave similar gas chromatographic results, but the ease of preparation and laboratory equipment necessary varied.

The lipid samples were refluxed in HCl and methanol for six hours to complete esterification when the method of Stoffel et al. (1959) was followed. This method required the greatest amount of laboratory equipment, but the reagents used were easily prepared.

When the method of de Man (1964) was followed, methyl esters could only be prepared after the free fatty acids present in the lipid samples had been neutralized. Complete esterification required holding the samples at 60 C for at least 24 hours. This method required the least amount of equipment, although the sodium methylate reagent is time consuming to prepare and requires caution during its preparation.

Esterification was complete in one-half hour using the boron trifluoride in methanol method of Morrison and Smith (1964).

The preparation of the boron trifluoride in methanol had to be done in a fume hood, and caution had to be taken. Once the reagent has been prepared, it can be stored without deterioration for at least a year.

Thus, the choice of method for the esterification of fatty acids involves consideration of the fatty acid composition of the fat samples (the presence of short chain fatty acids), the equipment available in the laboratory, the time available for preparation and the time allowed for the actual esterification.

E. Fatty Acid Composition of Feed Lipids

The fatty acid composition of the fat extracted from sow's gestation ration was similar to that of the fat extracted from the lactation ration. The lipids were much the same in appearance, and their saponification values were close. The major fatty acids present in the feed lipids were the same as those in the porcine fats, but the amounts of each fatty acid varied in the feed lipids from those of the porcine samples.

F. Glyceride Structure of Porcine Lipids

From a study of Tables 6 through 9, it is evident that there was a greater amount of variability between replicates

in the fatty acid composition of the purified mono-, di- and triglycerides after hydrolysis, than was observed in the fatty acid composition of the original triglycerides. This variability between replicates is shown more clearly in Tables 11 through 14, specifically in the percentage of a particular fatty acid esterified at the 2-position. The body fat samples studied seem to be more constant than the colostrum and milk samples. The averages of the four replicates studied (Tables 10 and 15) indicate the general trends followed by the individual samples.

There was little difference between the fatty acid composition of the original triglycerides before hydrolysis and the triglycerides obtained after 15% hydrolysis, except in the case of body fat. When the fatty acid composition of the original triglycerides and the residual triglycerides after hydrolysis are similar, no significant preferential hydrolysis among the fatty acids at the 1- and 3- positions has occurred (Jack et al., 1963). A decrease in the amount of stearic acid in the residual triglycerides compared with the amount present in the original triglycerides of the body fat samples would indicate some preferential hydrolysis of stearic acid at the external positions in these glycerides.

The fatty acid composition of sow's colostrum, milk and body fat before hydrolysis and of the purified glycerides after

hydrolysis indicates that the general characteristics of porcine glyceride structure (palmitic acid esterified at the 2-position, and oleic and linoleic acids esterified at the 1- and 3-positions), established from the studies on depot fat and lard, are present in both colostrum and milk fat. Fatty acids which show an increase from the original triglyceride to the 2-monoglyceride, are assumed to be preferentially esterified at the 2-position on the glycerol molecule. Conversely, those acids which decrease from the original triglyceride to the 2-monoglyceride, are esterified at the external or 1- and 3-positions.

The increase of palmitic acid from the triglycerides to the 2-monoglycerides of the fat samples, and the decrease of oleic and linoleic acids in the 2-monoglycerides of these samples are outstanding characteristics of porcine body fat and lard. These trends were evidenced in all of the porcine lipid samples studied. There was also an increase in myristic acid in the 2-monoglycerides and a decrease in stearic acid. Capric and lauric acids seem to increase from the triglycerides to the 2-monoglycerides of the two milk fats studied, although with the small amounts present, precision in the estimation was not too high. The presence of capric acid in the monoglycerides of colostrum and body fats indicates that this acid is present in the triglycerides of these samples at levels

below detection by the gas-liquid chromatograph used, but increased amounts of the acid in the 2-monoglycerides enable its detection in the monoglycerides. Lauric acid was not detected in either the original triglycerides or the 2-monoglycerides of colostrum and body fat. Gadoleic acid, although present in very small amounts in most cases, seemed to decrease in the 2-monoglycerides. Variability was observed in palmitoleic and arachidic acids, making it difficult to note distinct increases or decreases in these acids.

The proportion of each fatty acid at the 2-position in the triglycerides of the fat samples studied (Tables 11-15) clearly indicates which fatty acids are preferentially esterified at the 2-position and which are esterified at the 1- and 3-positions. A value of 33.3% would indicate no preferential esterification of a particular acid at either the internal or the external positions. Smith et al. (1965) have suggested that less than 28% of a particular fatty acid esterified at the 2-position indicates that the fatty acid is located preferentially at the 1- and 3-positions and, conversely, more than 38% at the 2-position indicates preferential attachment of the acid at the 2-position. Although variability existed between replicates of the same sample source, the results indicated that there is a preferential incorporation of myristic and palmitic acids in the 2-position of all of the porcine fat samples studied. Stearic, oleic,

linoleic and gadoleic acids seem to be esterified at the external positions of all of the porcine glycerides studied. The proportion of palmitoleic acid at the 2-position varied between samples sources, but the proportion at the 2-position was quite constant between replicates. From the results, palmitoleic acid seemed to be randomly distributed in all of the glycerides studied with the possible exception of body fat, where there may be a preference for palmitoleic acid at the 2-position.

The proportion of arachidic acid at the 2-position varied between sample sources and between replicates. This could be a reflection on the amount of arachidic acid present in the fat samples. Results indicated that the distribution of arachidic acid on the glycerides examined may be random; the high value obtained for milk glycerides from the first week of lactation may not be significant, because as seen in Table 13, with the small amounts present, precision in the estimation was not high.

General patterns of glyceride structure, concerning the incorporation of fatty acids into internal and external positions of all the porcine glycerides studied, can be obtained from the data in Tables 6 through 16, but specific differences in structure between colostrum, milk and body fat glycerides

cannot be observed because of the variability between replicates.

By grouping the major fatty acids according to unsaturation, more information can be obtained from the data available. Results obtained after determining the percentage of saturated fatty acids at the 2-position (Table 16), showed that sow's colostrum and body fat have a higher proportion of saturated fatty acids at the 2-position than do sow's milk fat. The fat samples from milk during the first and third weeks of lactation were quite similar, and contained lower amounts of saturated fatty acids in the 2-position. These results do not agree with the general trends reported by Freeman et al. (1965) in their study on milk and body fats from various species. Porcine milk fat was not included in their investigations, but these authors found a lower proportion of saturated fatty acids at the 2-position of body fats than in their corresponding milk fats. It is well-known, however, that the structure of porcine glycerides is unique, and so this additional deviation from most species is not surprising. When comparing porcine glycerides to other animal glycerides, it is found that porcine milk fat is closer in its structure to the milk fat of other animals than is the structure of porcine body fat to the structure of body fat from other animal species.

Results from the calculation of glyceride types according to the method of Vander Wal (1960) (Table 17) indicate that

some differences may exist in the types of glycerides present in colostrum, milk and body fats. The calculations are based on the saturated fatty acid composition of the original fat and that of the 2-monoglycerides after hydrolysis. The data obtained follow the same pattern as outlined previously in the literature (Vander Wal, 1960; Coleman, 1961; Coleman, 1965).

Calculations indicated that colostrum fat had a structure more like body fat than milk fat, and that milk fats from the first and third weeks of lactation were similar. The similarity in glyceride structure between colostrum and body fat and between milk fat from the first week of lactation and that from the third week is in agreement with the corresponding fatty acid compositions. Higher amounts of GS_3 and GS_2U were present in the milk fats than in the colostrum and body fats, but the latter fats contained more GSU_2 . However, when considering the GU_3 content of the samples, colostrum contained more of this glyceride type than any of the other samples. The distribution of GS_2U isomers, SUS and SSU , in the colostrum, milk and body fat samples, followed the distribution of GS_2U . For example, milk fat contained a larger amount of GS_2U than colostrum and body fat, and this was reflected in the larger amounts of its two isomers. The isomers of GSU_2 did not follow similar patterns. Colostrum and body fat contained greater amounts of USU than milk fat, but less UUS .

An elaboration of the differences between the glyceride structure of porcine colostrum, milk and body fats could be obtained by using such techniques as oxidation and chromatography as outlined in the review of literature.

G. Preferential Incorporation of Fatty Acids in the 2-Position
According to Unsaturation and Chain Length

Brockerhoff et al. (1966) studied the positional distribution of fatty acids in the triglycerides of animal depot fat and concluded that the distribution between the internal and external positions was governed by chain length and unsaturation. The shorter and the more unsaturated fatty acids show a greater tendency to occupy the internal position. These authors maintained that this rule also applies to porcine fat, but in these glycerides the influence of chain length overrides that of unsaturation. To determine whether shorter chain fatty acids or saturated fatty acids have a greater preference for the internal position on porcine triglyceride molecules, comparisons were made between short and long chain fatty acids at the 2-position, and between saturated and unsaturated fatty acids at the 2-position (Table 18). These results show that there seems to be a higher proportion of short chain fatty acids than saturated fatty acids at the 2-position in all of the fat samples studied. Also, there is

a greater difference between the incorporation of short and long chain fatty acids than between the incorporation of saturated and unsaturated fatty acids at this position. These results are influenced by the fact that stearic acid, a long chain saturated acid, was preferentially incorporated into the external positions of the glycerides studied; the percentage of saturated fatty acids at the 2-position would be lowered because of this acid. Thus, it seems that the incorporation of fatty acids into the 2-position of porcine glycerides is influenced more by the chain length of the fatty acid than the degree of unsaturation, and that there is a greater preference at the 2-position for fatty acids containing fourteen and sixteen carbon atoms for saturated fatty acids generally. These results support the theory of Brockerhoff et al. (1966), that short chain fatty acids are preferentially incorporated into the internal positions of porcine glycerides.

In support of the results from Table 16, Table 18 shows that there is a greater incorporation of saturated fatty acids in the 2-position of sow's colostrum and body fat than in sow's milk fat. From Table 18, it would seem that short chain fatty acids are also more readily incorporated into the 2-position of colostrum and body glycerides than in milk glycerides.

H. Glyceride Structure of Feed Lipids

The fatty acid composition of the fats extracted from sow's gestation and lactation ration before hydrolysis, and the purified glycerides obtained after 15% hydrolysis with pancreatic lipase (Table 19), indicates that the two rations have not only similar fatty acid compositions, but also similar glyceride structures.

The fatty acid composition of the original triglycerides, and that of the residual triglycerides after hydrolysis, varied more than in the similar study on porcine glycerides. There was a decrease in palmitic, stearic, linoleic and arachidic acids from the original triglycerides to the residual triglycerides, and an increase was observed in the amount of oleic and linoleic acids in the residual triglycerides from the original ones. This would indicate a preferential hydrolysis of those acids which decreased in the residual triglycerides (palmitic, stearic, and linolenic and arachidic acids). From the results, it seems that myristic, oleic and linoleic acids are situated at the 2-position of the feed glycerides, and palmitic, stearic, gadoleic, linolenic and arachidic acids at the 1- and 3-positions of the glycerides. Palmitoleic acid is randomly distributed between the internal and external positions. This type of distribution closely follows the general trend for plant lipids

(Mattson and Volpenhein, 1961b).

I. The Influence of the Dietary Lipids on Sow's Colostrum,
Milk and Body Tissue Lipids

The sows used in this study were on standard diets containing approximately 3% fat. Considering the alterations which dietary fat undergoes before being utilized, it is unlikely that this diet would influence either the fatty acid composition, or the glyceride structure of the fat samples studied. An animal on a diet containing only 3% fat, would synthesize its lipids de novo, and the resulting lipids would be more characteristic of that particular animal than of the dietary fat.

Flanzy et al. (1965) reported that the glyceride structure of dietary fat was not retained in the depot fat of swine. Dahl and Persson (1965) stated that in milk fat, more of the character of dietary fat is reflected because the milk fat is more rapidly formed. It would be interesting to carry out a study in which sows were fed a high fat diet to see if more retention of dietary glyceride structure was possible in the colostrum and milk fat than body fat. Because they are non-ruminants, sows are ideal animals to work with in such a study. The influence of dietary fat on animal fats is more

easily followed in the non-ruminant because less alteration of dietary fat occurs in the absence of rumen bacteria. Also, sow's milk fat contains no short chain fatty acids, and thus the technique of lipase hydrolysis can be used to determine the glyceride structure.

Part II Fatty Acid Composition and Glyceride Structure of
Lipid Samples from Piglets on a High Fat Diet;
Comparison of Five Sampling Sites

A. Saponification Values

The saponification values of the body tissue fat from piglets on a high fat diet, (Table 20) were within the range reported for lard by Mehlenbacher (1960), and were close to the saponification value determined in Part I for sow's body fat.

B. Fatty Acid Composition of Body Tissue Lipids from
Various Sites

The fatty acids present in the body fat of the piglets on a high fat diet were the same as those found in the body fat of the sows in Part I. The amounts of each fatty acid present were closer to the amounts present in the body fat of sows on a

diet containing 15% stabilized tallow reported by de Man and Bowland (1963), than to the amounts present in the sows on a standard diet (Part I; de Man and Bowland, 1963). The fats from the body tissues of the piglets on the high fat diet contained greater amounts of oleic acid and group B acids, and less linoleic acid; the decrease in palmitic acid found by de Man and Bowland (1963) was not noted in the piglet fat samples.

An analysis of variance of the component fatty acids in the fat samples, and the application of Duncan's new multiple range test to the data (Table 22) brought out the differences in composition between sampling sites. All of the fatty acids present except myristic, arachidic and gadoleic acids showed highly significant differences between sites. In most cases, kidney fat was found to be significantly different from the other sample sites; this is particularly evident in palmitic, stearic and oleic acids. These results were not surprising. Kidney fat represented the only organ fat studied, whereas all of the other sampling sites were adipose tissue. The kidney fat is thus associated with a highly differentiated organ and would be undergoing a more rapid turnover than the fat in the other sites. This may also mean that the diet may have more of a direct influence on the fatty acids of kidney glycerides.

When considering the depot fats taken from the shoulder and the loin, there seemed to be more difference between samples taken from the inner and outer layers than between samples taken from the shoulder and the loin. This was best illustrated with stearic and oleic acids; there was no statistical difference between outer shoulder and outer loin, or between inner shoulder and inner loin, but the difference between outer and inner sites was highly significant. This trend could be seen in the other fatty acids analyzed. In palmitoleic acid, outer shoulder and outer loin were not significantly different from each other, but were different from the other sites analyzed; inner shoulder and inner loin belonged to the same subset, and were not statistically different from each other. The same observations could be made in the analysis of linoleic acid. The amount of palmitic acid did not vary in the shoulder and loin sites, whether taken from the inner or outer layers, but the amount of palmitic acid in kidney fat was significantly different from the amount of this acid in the other sites.

It can be seen from the data obtained in this study, that greater differences between sites were obtained by such acids as stearic and oleic which were readily synthesized in the body, than by linoleic acid which probably had been deposited from dietary sources. These observations are in

agreement with those of Imaichi et al. (1965).

The fatty acid composition of the inner shoulder and inner loin fats remained intermediate between the fatty acid composition of the kidney fat and the fatty acid composition of the outer shoulder and outer loin fats. Specifically, greater amounts of palmitic and stearic acids and lesser amounts of oleic acid were found in kidney and inner body fats than outer body fats. These results agree with those of Leat et al. (1962) and Sink et al. (1964) who found more saturated fatty acids in inner back fat, perirenal and perinephric tissues.

C. Glycerides Structure of Body Tissue Lipids from Various Sites

A study of Tables 23 through 27 reveals that much less variability between replicates existed in these data than in the hydrolysis data of Part I. A possible exception in Part I may be sow's body fat which was fairly constant. Less variability seems to occur then, in data concerned with body fat samples than data concerned with colostrum and milk fat samples. This could indicate that less variability exists in the glyceride structure of body fats than of colostrum and milk fats, which are more rapidly formed in the animal body.

There was a decrease in the amount of stearic acid and an increase in the amounts of oleic and linoleic acids from the original triglycerides to the purified triglycerides obtained after 15% hydrolysis. These increases and decreases were the greatest in kidney fat, and indicate that pancreatic lipase may be preferentially hydrolyzing stearic acid at the external positions of the glycerides (Jack et al., 1963). A similar situation was found for the sow body fat and the feed lipids, but not for the colostrum or milk fats. This preferential cleavage found only in some fats could be caused by the physical structure of the actual fat globule being hydrolyzed, as proposed by Brockerhoff (1966). One would expect to find a variation in the physical structure of fat globules from milk and body tissues.

The general characteristics of porcine glyceride structure (high amounts of palmitic acid at the 2-position and high amounts of oleic and linoleic acids at the 1- and 3-positions) are evident in all of the samples studied. As observed in Part I, myristic and palmitic acids increased from the original triglycerides to the purified 2-monoglycerides obtained after hydrolysis. Palmitoleic acid increased in the monoglyceride fraction from the shoulder and loin fats, but decreased slightly in kidney fat. Stearic, oleic, linoleic, arachidic and gadoleic acids all decreased from the original triglycerides

to the residual 2-monoglycerides.

Tables 28 through 33 show the proportion of each fatty acid esterified at the 2-position. As mentioned previously, Smith et al. (1965) have established that less than 28% of a particular fatty acid esterified at the 2-position indicates that the fatty acid is located preferentially at the 1- and 3-positions, and more than 38% at the 2-position indicates preferential attachment of the acid at this position. The percentage calculated, indicated that myristic and palmitic acids were preferentially incorporated into the 2-position of the porcine glyceride samples studied. Stearic, oleic, linoleic, arachidic and gadoleic acids were esterified primarily at the 1- and 3-positions in the piglet samples. Palmitoleic acid seems to be preferentially esterified at the 2-position in the inner and outer shoulder, and inner and outer loin fats, but this acid seems to be located at the 1- and 3-positions in kidney fat.

When the preferential incorporation of fatty acids into the 2-position of the porcine glycerides was compared on the basis of unsaturation or chain length (Table 34), there seemed to be more of a preference for the shorter chain fatty acids (myristic, palmitic and palmitoleic) than for the saturated fatty acids at the 2-position of the glycerides studied. These

results agree with those in Part I.

Kidney fat showed a lesser tendency than the other fats to esterify saturated fatty acids and shorter chain fatty acids at the 2-position. This can be seen from the results in Table 34. The proportion of saturated and shorter chain fatty acids at the 2-position of shoulder and loin glycerides was constantly higher than in kidney glycerides; this could mean that kidney glycerides may have a different structure from shoulder and loin glycerides.

Results from the calculation of glyceride types according to the method of Vander Wal (1960) (Table 35) indicated that some differences may exist in the types of glycerides present in the piglet fats from the various sample sites. From the calculations, kidney fat contained higher amounts of GS_3 and GS_2U , but lower amounts of GSU_2 and GU_3 than fats from the shoulder and loin. The isomers of GS_2U , SUS and SSU , were present in greater amounts in the kidney glycerides. Kidney fat contained the least amount of the isomer USU in comparison to the other sites, but little difference existed between sites as far as their UUS content was concerned.

A study of the glyceride types of inner and outer shoulder fat, and inner and outer loin fat, revealed that inner shoulder

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and inner loin fats contained more GS_3 and GS_2U than outer shoulder and outer loin fat. The amount of the two isomers of GS_2U was also greater in the inner fat samples. Outer shoulder and outer loin fats seemed to contain more GU_3 , but the amount of GSU_2 and its isomers, USU and UUS , in the shoulder and loin samples was varied. It is interesting to note that the three sites, kidney, inner shoulder and inner loin, which contained the higher amounts of saturated fatty acids, also contained higher amounts of GS_3 and GS_2U .

Both fatty acid composition and glyceride type distribution of the piglet fats differed from the data of Chacko and Perkins (1965). The type of diet given to the pigs in the above study was not described by the authors, but their results seem to be closer to the results obtained in Part I of this thesis, when the body fat of sows on a standard diet was examined. Other differences such as the age and breed of the pigs used in the two studies, and the method used to determine the glyceride structure of the fat could influence the results obtained. Although Chacko and Perkins used the pancreatic lipase hydrolysis technique to examine glyceride structure, they determined the composition of the fatty acids at the 2-position by assigning the free fatty acids liberated by hydrolysis to the 1- and 3-positions, and calculating the composition of the fatty acids at the 2-position from the

composition of the original triglycerides. In this thesis, the fatty acids present in the 2-position were determined by examining the purified 2-monoglycerides obtained after hydrolysis; this has been suggested to be a more reliable method (Coleman, 1963).

Nevertheless, the general conclusions drawn from both sets of data are quite similar. Chacko and Perkins concluded that both fatty acid composition and glyceride structure varied, depending on the position within the animal from which the depot fat was obtained. Results of this study indicate that there was variation in fatty acid composition with the sampling site, but there were greater differences between some sampling sites than others. For example, there was a greater difference between kidney fat, and outer loin fat, than between outer loin fat and outer shoulder fat. The glyceride structure of fats taken from different sites also seemed to vary, and again, greater differences seemed to exist between some sampling sites than others. As with fatty acid composition, greater differences in the glyceride type distribution seemed to occur between the kidney glycerides, and the shoulder and loin glycerides. Also, there were greater differences between outer and inner fats than between samples taken at the same depth but at different locations.

D. Fatty Acid Composition and Glyceride Structure of Tallow Lipids

Stabilized tallow, which made up 20% of the ration given to the piglets in this study, contained the same fatty acids as were found in the body fats examined, but the amounts of the acids present in the tallow varied from the amounts reported in the piglet fat (Table 21). The fatty acid composition of the original tallow triglycerides, and the composition of the purified mono-, di- and triglycerides after hydrolysis are listed in Table 36. Generally, the tallow contained greater amounts of myristic and stearic acids and lower amounts of oleic and linoleic acids than the piglet glycerides. The fatty acid composition of the tallow was more like that of piglet's kidney fat than that of the shoulder and loin fats.

A comparison of the original tallow triglycerides and the residual triglycerides showed that the amount of stearic and palmitic acids present in the triglycerides obtained after hydrolysis was less than the amount present originally; there was also an increase in the amount of oleic and linoleic acids present in the residual triglycerides. These discrepancies between the original and residual triglycerides were also noted in the feed lipids, and to a lesser extent in the sow body fat and piglet fats, and a possible explanation of these inconsistencies was given in the discussion concerning these fats.

The hydrolysis data from Table 36 indicates that myristic, palmitic, palmitoleic and group A acids were preferentially esterified at the 2-position; stearic, oleic and group B acids were preferentially esterified at the 1- and 3-positions, whereas arachidic and gadoleic acids were randomly distributed. From the data available, it is difficult to say whether linoleic acid is distributed randomly or esterified preferentially at the 1- and 3-positions.

E. The Influence of Dietary Lipids on Piglet Glycerides

Definite differences were observed between the fatty acid composition of the body fats of piglets given a high fat diet containing 20% stabilized tallow, and the fatty acid composition of the body fat of sows on a standard diet. As mentioned previously, the piglet body fats contained higher amounts of oleic and group B acids, and a lower amount of linoleic acid.

A comparison of the tallow lipids and the standard gestation and lactation ration lipids reveals that tallow contained greater amounts of myristic, palmitic, stearic and oleic acids, and lesser amounts of linoleic acid than the standard rations. The increased amount of oleic acid, and the decreased amount of linoleic acid in the tallow lipids was reflected in the body fats of the piglets which had higher amounts of oleic acid and lower amounts of linoleic

acid than body fat of sows on a standard diet. Although there were greater amounts of the saturated fatty acids, myristic, palmitic and stearic, in the tallow, there were no increases of these acids in the body fats of the piglets. It seems then, that unsaturated dietary fatty acids may influence the composition of the depot fat more than saturated fatty acids. This was indicated in the data of Christensen et al. (1963) and Chung and Lin (1965) which showed that unsaturated fatty acids were more directly deposited, and saturated fatty acids were transformed before deposition.

As mentioned before, tallow contained more myristic, palmitic and stearic acids, and less oleic acid than the standard rations, and piglet's kidney fat followed these trends more closely than the outer and inner shoulder, and outer and inner loin. It would be difficult to say with the limited data available here, if kidney fat reflected the fatty acid characteristics of the diet more than the other sites studied. More data would be necessary from animals on a variety of diets before this could be considered.

The general characteristics of both the piglets' glyceride structure and the sows' glyceride structure were similar, in that myristic and palmitic acids were esterified preferentially at the 2-position of the glycerides, and stearic,

oleic and linoleic at the 1- and 3-positions. A calculation of the amount of each glyceride type (Vander Wal, 1960) revealed that there may be some differences in glyceride structure between the piglet glycerides and the sow glycerides. A direct comparison of the glyceride types present in sow's body fat and piglet's body fat cannot be made on the basis of their diets alone because factors such as the age and breed of the two groups of pigs would also have to be considered. Nevertheless, the glyceride types calculated by Chacko and Perkins (1965) seemed to be closer to the glyceride types of the sow body fat than those of the piglet body fat, indicating that the diet may influence glyceride structure to some extent. These variations in glyceride structure may be direct reflections of the variations in fatty acid composition induced by the diet, rather than a direct influence of specific structures in dietary fat which have been retained in the animal body fat. Privett et al. (1965) maintained from their studies that although the triglyceride types present did not change, the amounts of each triglyceride type varied considerably in relation to the fatty acid composition of the diet.

From the literature reviewed, (Brockerhoff et al., 1964; Clément et al., 1965a; Flanzky et al., 1965; Privett et al., 1965) it seems that little retention of dietary glyceride structure occurs in the mammalian fats. It would be impossible

from the data of this thesis, to determine any retention of the original dietary structure in the structure of the porcine fats. Experiments using glycerides labelled with specific fatty acids may reveal the information necessary to show whether retention of dietary glyceride structure occurred to a greater extent in some body sites than others.

Part III Identification of Arachidic Acid

Plotting the log of the retention time versus carbon number and retention time versus number of double bonds (Figures 8 and 9) indicated the possibility that the peak, eluted after methyl linoleate and before methyl gadoleate, could represent either methyl linolenate or methyl arachidate. This possibility was verified when standard linolenate and arachidate had the same retention time on the diethylene glycol succinate column as the unknown peak.

Because the unknown peak was usually of the same size or larger than the peak representing gadoleic acid, and the amount of C_{18} fatty acids was much greater than the amount of C_{20} fatty acids, there was a great deal of difference in the magnitude of the 3:2 ratio and the 4:1 ratio (Table 37). In most of the samples from the sow and piglet experiments, the 3:2 ratio, established from chromatograms of the polar

column was very similar to the ratio obtained with the nonpolar column. These results indicate that the unknown peak represents arachidic acid and not linolenic acid. Trace amounts of linolenic acid ($<0.2\%$) in the sample could be present and not detected by the method used in this study. The evidence obtained from these chromatograms plus the negative results of the linolenic acid test indicate the presence of arachidic acid in the porcine glyceride samples.

Results from the gestation and lactation glycerides are not as straightforward as those from the porcine glycerides. The ratio established from the silicone column lies between the 3:2 and 4:1 ratios derived from the polar column. This indicates that these rations contain both linolenic and arachidic acids. The quantitative determination of each would be difficult to carry out without further and extensive experimentation. For the purposes of this project, the identification of the presence of both linolenic and arachidic acids was sufficient.

A mixture of both linolenic acid and arachidic acid would explain the negative results obtained when the linolenic acid test was carried out on the feed lipids. A minimum amount of linolenic acid is required to obtain positive results (White and Brown, 1949), and this minimum would not have been present

if the unknown peak represented a mixture.

The tallow, which was incorporated into the diet of the piglets, gave results similar to those of the porcine glycerides, in that the presence of arachidic acid was confirmed.

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Table A-1. Names and abbreviations of fatty acids.

Molecular formula	Common name	Systematic name	Abbreviation used in text
$C_{10} H_{20} O_2$	Capric	Decanoic	10:0
$C_{12} H_{24} O_2$	Lauric	Dodecanoic	12:0
$C_{14} H_{28} O_2$	Myristic	Tetradecanoic	14:0
$C_{16} H_{32} O_2$	Palmitic	Hexadecanoic	16:0
$C_{16} H_{30} O_2$	Palmitoleic	Hexadecenoic	16:1
$C_{18} H_{36} O_2$	Stearic	Octadecanoic	18:0
$C_{18} H_{34} O_2$	Oleic	Octadecenoic	18:1
$C_{18} H_{32} O_2$	Linoleic	Octadecadienoic	18:2
$C_{18} H_{30} O_2$	Linolenic	Octadecatrienoic	18:3
$C_{20} H_{40} O_2$	Arachidic	Eicosanoic	20:0
$C_{20} H_{38} O_2$	Gadoleic	Eicosenoic	20:1
$C_{20} H_{36} O_2$		Eicosadienoic	20:2
$C_{22} H_{44} O_2$	Behenic	Docosanoic	22:0

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